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Observations on a Chemically Induced Chicken Tumor Containing an Antigen Related to That of a Leukosis Sarcoma Agent*

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Cell-free transmissions of chemically induced sarcomas of fowls were reported by McIntosh (49), by Haddow (36), who, however, could not repeat his first experiments (37), and by McIntosh and Selbie (50, 63). Many other investigators, however, have failed to transmit such tumors by cell-free material (25, 51, 53, 55, 56, 59, 68). A close immunological relationship between the agent of Rous sarcoma I and constituents of chemically induced tumors was, however, indicated by neutralization experiments (7, 25, 26). Andrewes (7) transferred to pheasants a nonfilterable chicken sarcoma produced by tar; these pheasants developed neutralizing antibodies to the agent of Rous sarcoma I. Foulds (25, 26) injected rabbits with either tumor particles, extracts, or filtrates from a chicken sarcoma induced by 1,2,5,6-dibenzanthracene; the immune sera neutralized the agent of Rous sarcoma I. Recent observations show that it is possible to concentrate by high speed centrifugation both the agents of filterable chicken tumors (16, 41) and several normal heavy components of tissues, such as enzymatic substances (42, 64), organ- and species-specific antigens, and heterogenetic antigens (30, 31, 38, 39). On the basis of this experience, the relation between filterable and nonfilterable chicken tumors has been reinvestigated.

Sarcoma 13 has been used as a source of a filterable tumor agent. This agent is able to cause sarcomas as well as endotheliomas and erythroleukosis or myeloleukosis (67); it is referred to in this paper as agent 13. Kabat and Furth have shown that rabbits repeatedly injected with high speed sediment from sarcoma 13 develop neutralizing antibodies to agent 13 (43). The experiments to be discussed show the presence of an antigen related to or identical with that of agent 13 in the nonfilterable sarcoma 16, originally induced by methylcholanthrene.

OBSERVATIONS ON THE CHEMICALLY INDUCED SARCOMA 16

ORIGIN OF SARCOMA 16

Sarcoma 16 originated in this laboratory in a barred Rock chicken that had been injected with methylcholanthrene in the breast when 20 days old; this chicken received 6 days later an intravenous injection of erythroleukotic plasma, strain 1 (27). After 80 days a sarcoma developed at the site of injection of the methylcholanthrene, grew for 7 months, and metastasized to the lungs, liver, and heart. This chicken showed no evidence of erythroleukosis.

Of the 8 chickens injected in the above experiment with methylcholanthrene and strain 1 agent, 4 developed sarcomas and one erythroleukosis. In 8 chickens injected with methylcholanthrene alone, 4 sarcomas appeared. Of 6 chickens injected with leukotic plasma alone, 1 developed erythroleukosis. This experiment failed to show a reciprocal enhancement of activity between the agent of strain 1 and methylcholanthrene.

TRANSMISSION OF SARCOMA 16 IN CHICKENS

Sarcoma 16 has been transferred in chickens for 27 generations by intramuscular injection. No signs of erythroleukosis or myeloleukosis have been found in any of the injected chickens, even though 190 of these were kept for more than 2 months. It seems unlikely that the leukosis agent 1 has been carried

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in association with sarcoma 16 throughout these transfers without giving evidence of its presence. A suspension of cells from sarcoma 16 failed to produce leukosis or tumors in 7 chicks injected intravenously when 5 to 7 days of age.

Barred Rock chickens were injected intramuscularly in both breasts, or in both breasts and thighs, with about 0.15 ml. of a suspension of sarcoma 16 particles in Tyrode's solution. More than 80 per cent of the chickens injected between the ages of 2 and 50 days developed tumors (Table I). The grafted sarcomas appeared after 1 or 2 weeks. They were composed of densely packed spindle-shaped cells with little intercellular substance; atypical nuclei were frequent and mitoses numerous (Figs. 1 and 2). Lymphocytic infiltration was occasionally found. Some necrosis occurred when the tumor became large. In young chickens sarcoma 16 frequently invaded the skin, mediastinum, peritoneum, and liver by direct extension and sometimes metastasized to the kidneys, liver, and lungs (Figs. 3 and 4). In older chickens the tumor frequently regressed when small. Several foci of tumor usually regressed simultaneously. The frequency of regressions was directly related to the age of the animals at the time of injection (Table I).

Tumor stored for several months at −60° C. transmitted the disease as readily as fresh sarcoma particles. Some chicks were irradiated with 250 to 400 r before injection with sarcoma 16 cells. This treatment may decrease the tendency of the transfers to regress; however, only the figures obtained with birds 16 to 50 days of age differed significantly (Table I).

In 3 chickens a tumor developed several months after injection. These unusual tumors will be designated as *late sarcoma 16.* Duran-Reynals recently described chicken and duck tumors appearing late after heterologous transfers of Rous sarcoma I and containing a modified form of the agent (22). Because of the contradiction existing between the nonfilterability of sarcoma 16 and its immunological

TABLE I: RELATION OF AGE TO SUSCEPTIBILITY TO SARCOMA 16

Age of chickens at injection, days	Number of chickens injected	Number of chickens positive	Chickens positive, per cent	Number of chickens died from sarcoma	Number of chickens with sarcoma that regressed	Chickens died from sarcoma, per cent
A. CHICKENS INJECTED WITH UNFROZEN SARCOMA CELLS						
2 to 5	88	85	97	27	8	77
6 to 10	50	43	86	6	14	30
11 to 15	74	70	95	8	31	21
16 to 50	108	87	81	4	55	7
Above 50	2	0	0	—	—	—
B. CHICKENS INJECTED WITH SARCOMA CELLS STORED AT −60° C.						
2 to 5	14	14	100	9	0	100
6 to 10	32	25	78	4	7	36
11 to 15	4	2	50	0	3	0
16 to 50	16	12	75	0	10	0
Above 50	9	3	33	0	2	0
C. CHICKENS IRRADIATED BEFORE INJECTION WITH SARCOMA CELLS						
2 to 5	2	2	100	1	0	100
6 to 10	17	17	100	8	7	53
11 to 15	6	5	86	0	5	0
16 to 50	10	9	90	3	3	50
D. CHICKENS INJECTED WITH "LATE SARCOMA" CELLS						
2 to 5	6	6	100	4	1	80
11 to 15	4	3	75	0	3	0
16 to 50	11	8	73	2	4	33
E. TOTAL						
2 to 5	110	107	97	41	9	82
6 to 10	99	85	86	18	28	39
11 to 15	88	80	91	8	42	16
16 to 50	145	116	80	9	72	11
Above 50	11	3	27	0	2	0

The percentage of regression is based on the fate of the grafted animals that were not killed while they bore tumors. As the fastest growing tumors were taken for transfer or storage, the figures of Table I may indicate too high a percentage of regression.

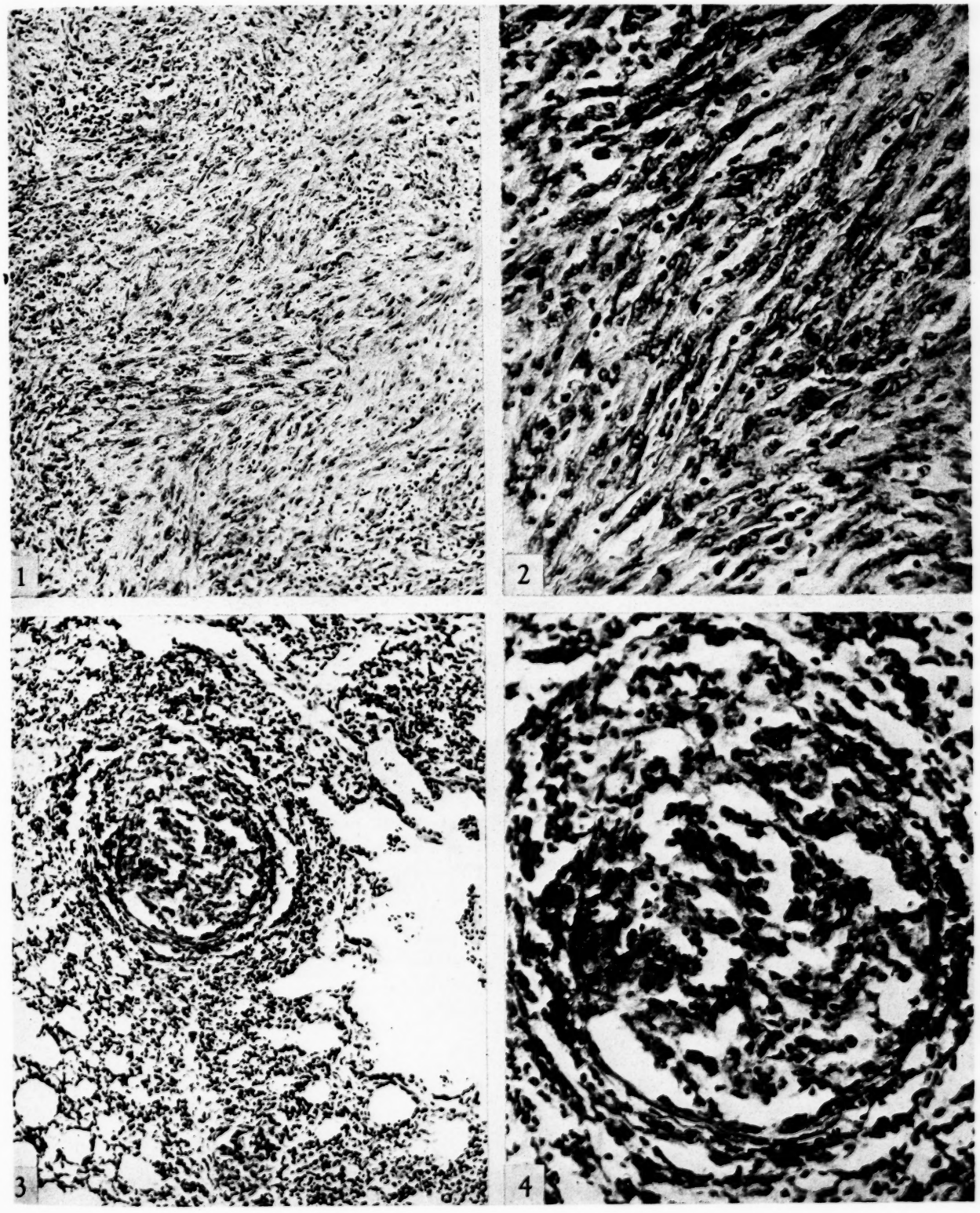

FIG. 1.—Sarcoma 16 growing in the breast of chicken 787. There is some lymphocytic infiltration. Hematoxylin–eosin stain. Mag. × 180.

FIG. 2.—Sarcoma 16. High power view of the same specimen as Fig. 1. Numerous mitotic figures are visible. Hematoxylin–eosin stain. Mag. × 450.

FIG. 3.—Metastatic sarcoma 16 cells growing in a small artery of the lung and breaking through its wall. A similar process can be seen in an arteriole in the lower part of the figure. The serum of this chicken, No. 787, contained no neutralizing antibodies to agent 13. Hematoxylin–eosin stain. Mag. × 180.

FIG. 4.—Same as Fig. 3. Mag. × 450.

Sarcoma 16 is also illustrated in a publication of Burk and his associates (11).

behavior suggesting the presence of a filterable agent, it was of interest to investigate whether the late sarcoma 16 represented a modified tumor. However, except for the size, the late sarcomas 16 did not differ in their gross and microscopic appearance from the usual sarcomas 16. The 3 late tumors developed at sites that previously had been injected with cells; all 3 remained localized.

Chicken 462 was unsuccessfully injected with sarcoma 16 cells at the age of 4 days and was reinjected when 65 days old. After more than 1 month a tumor developed in the twice injected leg; it measured $35 \times 25 \times 20$ mm. when the bird was killed at the age of 4 months.

Chicken 576 was injected in both breasts when 14 days old; 2 large tumors ($40 \times 10 \times 10$ mm.) developed and regressed within 1 month. When the chicken was 3 months old a mass appeared in the right breast; it reached a size of $140 \times 75 \times 65$ mm. at the time of death 2 months later.

Chicken 596 received cells in both breasts when 23 days old. A 1 mm. nodule appeared and regressed within 10 days. A mass developed in the opposite breast 34 days after injection and reached a size of $20 \times 10 \times 8$ mm. This animal died of intercurrent infection at the age of 4 months while its tumor was regressing ($8 \times 3 \times 3$ mm.). The tumor and the liver contained numerous polymorphonuclear leukocytes, myelocytes, and lymphocytes, and the bone marrow was hyperplastic but not leukemic. A suspension of cells from the moderately enlarged spleen was injected into the veins, or into the muscles, of 8 chicks 7 days of age; no leukosis or tumors were obtained after 70 days.

The transfer of particles from 2 late sarcomas 16 (Nos. 462 and 576) gave rise to early sarcomas that regressed in the usual proportion according to the age of the recipients (Table I). One of the 50 day old chickens that had been injected with late sarcoma No. 576 died after 2½ months, bearing a large, slowly growing tumor similar to tumor No. 576; this transfer, however, started to grow in the recipient immediately upon inoculation. Ten birds were observed for 3 months following the injection of late sarcoma 16 particles; in 8 of these an early tumor had regressed, and in the 2 others the transfers had been unsuccessful; none of them developed a late tumor.

Since transfers from late sarcoma 16 behave similarly to those made with particles of the usual sarcoma 16, a modification of the cells or of a hypothetical filterable agent cannot account for the appearance of the late tumors that we observed. They differ from those described by Duran-Reynals (22) but are comparable with the spontaneously recurring Rous tumor I recently observed by Carr (12). Most of the recurring tumors described by this author occurred in a breed of low susceptibility to the agent of Rous sarcoma I. They could not be transmitted by filtrates into birds of normal susceptibility, but the filterable agent could be readily recovered from the tumors produced in normal birds by cellular grafts. Neither cellular grafts nor injection of filtrate of recurring tumors produced sarcomas in birds of the breed of low susceptibility. Thus Carr found no evidence of a modification of the agent. As the tumors recurred in a few instances at more than one inoculation site, he assumed that the recurrence of the tumors was due to a modification of the general resistance of the host. The neutralization experiments that will be described support the view that the appearance of late sarcomas 16 is related to the small amount of antibodies present in the sera of their hosts.

Failure of Attempts to Transmit Sarcoma 16 by a Filterable Agent

Murphy and Sturm described the failure of attempts to transmit sarcoma 16 by filtrate or by cells that had been damaged by x-ray (55). The following experiment shows that cells desiccated in the frozen state likewise are not infective.

Five 2 day old chicks were injected intramuscularly at each of 4 sites with lyophilized sarcoma 16 cells; no sarcomas were obtained in 100 days. After 3 months one of these chickens developed neurovisceral lymphomatosis (the ovary and cecum were infiltrated), and a lymphomatous nodule appeared in the skin of the uninjected wing shortly before death. Attempted transmission of the ovarian and skin tumors by intramuscular and intravenous injections failed in 10 chicks injected when 12 days old and killed 2 months later. It appears that the lymphomatosis was spontaneous. It is a common disease among adult chickens (28, 32); however, only one of all the chickens injected with sarcoma 16 cells developed fowl paralysis.

Transfers of Sarcoma 16 into Ducks

Duran-Reynals suggested recently that some paradoxical observations on bird tumors could be explained on the basis of heterologous infections (22). The newly hatched duck is a favorable recipient for heterologous transfers (22). Table II shows the results of cellular transfers of early and late sarcoma 16 (Nos. 462 and 576) into 1 day old ducks.

White Pekin ducklings were used 18 to 27 hours after hatching; in one transfer they were 49 hours old. One to 0.05 ml. (usually 0.2 ml.) of cell suspension was injected into both breasts. The sarcomas grew quickly and passages in ducklings were made at weekly intervals. In about one-half the ducks the

tumors regressed. Some ducks died with huge tumors. Extensions of tumors into the abdomen and chest were a frequent finding and in 2 animals metastases to the lungs were found. The growth of sarcoma 16 nodules in the liver was often associated with infiltration by myelocytes and polymorphonuclear leukocytes. No difference was observed between the behavior of transfers from late and early chicken tumors. No other tumors than sarcomas were found and there was no evidence of the presence of a neoplastic agent independent of the cells, such as endotheliomas arising at a distance in chickens bearing sarcoma 13 (67). No late tumors appeared in 14 ducks surviving the injection for 2½ to 3½ months.

Attempts were made to demonstrate a filterable agent in sarcoma 16 grown in ducks.

Table II: Transfers of Chicken Sarcomas 13 and 16 into 1 Day Old Ducklings

Material injected	Route of injection	Number of passages in ducklings	Number of ducklings injected	Number of ducklings positive	Ducklings positive, per cent	Number of ducklings died from sarcoma	Number of ducklings with sarcoma that regressed	Ducklings died from sarcoma, per cent
Sarcoma 16 cells from early chicken tumors	Intramuscular	I and II	9	8	89	4	2	67
Sarcoma 16 cells from "late" chicken tumors	Intramuscular	II and X	44	43	98	9	12	43
Extracts from 1st to 10th generation in ducks of "late" sarcoma 16	Intramuscular	—	9	0	0	—	—	—
Sarcoma 13 cells from chicken tumors	Intramuscular	I and II	12	11	92	4	4	50
Extract from chicken sarcoma 13	Intramuscular	—	3	0	0	—	—	—
" "	Intravenous	—	6	0	0	—	—	—
Extract from 2nd generation in ducks of sarcoma 13	Intramuscular	—	3	0	0	—	—	—
" "	Intravenous	—	5	0	0	—	—	—

Extracts from first, fourth, and tenth passages of late sarcoma 16 (No. 576) grown in ducks were prepared by triple extraction with equal volumes of saline; the cells were removed by two centrifugations at 3,000 to 4,000 r.p.m. for 30 minutes. A part of the extract was filtered through a Berkefeld V filter. One milliliter of the filtered or unfiltered extract was injected at each of 4 sites in newly hatched ducklings (29 to 31 hours old). The injected birds were kept for 3 months. No tumors were obtained (Table II). The extracts were extremely toxic when injected intravenously.

After passages through ducks, the sarcoma 16 cells were still infective for chickens; indeed, there is some indication that their virulence was enhanced: all except 1 of the 9 chickens 16 days old injected with sarcoma 16 particles from the fifth and tenth generations in ducks died with large tumors (Table I). The cell-free extract from the first transfer of sarcoma 16 in ducks was injected at 4 sites in 5 chicks 3 days old and failed to induce tumors within 3 months.

For comparison, heterologous transfers of the filterable chicken sarcoma 13 were made in newly hatched ducklings (Table II). Transfers of sarcoma 13 particles were about as successful as those of sarcoma 16. An extract made from sarcoma 13 grown in ducklings proved highly infective when injected into chicks.

Three chickens that received intravenously 1 ml. of the extract diluted 1:8 died after 8 to 18 days, showing the hemangioendotheliomas characteristic of agent 13 (67). Two-tenths of a milliliter of dilutions of this filtered extract in saline containing 10 per cent rabbit serum were injected intramuscularly in chickens. Sarcomas appeared at all 9 sites injected with dilution 1:20 after an average latent period of 8.5 days [1] and at all the 9 sites injected with dilution 1:20,000 after a mean latent period of 16.6 days.[1] Dilutions in saline were slightly less active. The activity of this extract from sarcoma 13 grown in ducks is about 100 times that of our best preparations of extract from sarcoma 13 grown in chickens (*cf.* preparation 679 used in the neutralization experiments). This observation may be helpful in further studies on agent 13.

Ducks are not susceptible to agent 13 (Table II). The extract from sarcoma 13 grown in ducks was innocuous to newly hatched ducklings that received doses of 1 ml. at dilution 1:1 or 1:8 in the muscles or veins. These animals were observed for 4 months. Amounts of an extract from chicken sarcoma 13 (preparation 679), regularly infective in chickens, did not produce tumors in ducks in 3 months.

[1] These chicks were examined twice weekly.

The use of a heterologous host thus failed to show the presence of a neoplastic filterable agent in the chicken sarcoma 16, whereas agent 13 could be recovered from sarcoma 13 grown in ducklings.

COMPLEMENT FIXATION TESTS WITH HIGH SPEED SEDIMENT FROM SARCOMA 16

Preparation of antigen.—Sarcoma 16 was extracted 3 times with a 0.0002 *N* solution of sodium hydroxide in physiological saline; after preliminary centrifugation at 3,000 r.p.m. the debris was removed by a 15 minutes' run at 8,000 r.p.m. The supernatant from this centrifugation was spun for 1 hour at 27,000 r.p.m. (39,000 to 77,500 times gravity). The sediment was resuspended in the neutral saline, but part of the high speed sediment failed to dissolve. The proportion of soluble material seemed to be higher when the operations were carried out quickly and in the cold. The total heavy material (preparation 127) was used for immunization, the soluble part only for complement fixation tests.

Immunization of rabbits and chickens.—The animals were bled before immunization to obtain serum. Two rabbits and one adult fowl (175 days old) were injected intravenously 3 times at 4 day intervals with a suspension of high speed sediment of sarcoma 16 containing 2 mgm. of protein calculated from the nitrogen content. Since test bleedings yielded rabbit sera with low complement fixation titers, the injections were resumed after an interruption of 15 days. Four additional injections were given at similar intervals; in each the material injected contained 3 mgm. of protein. The animals were bled 6 days after the last injection. An additional 3 rabbits and 2 adult fowls were given alum-precipitated heavy material from sarcoma 16 in similar amounts.

TABLE III: HIGH SPEED SEDIMENTABLE MATERIAL IN SARCOMA 16 AND SPLEEN

Preparation	Amount of tissue (wet weight), gm.	Supernatant after centrifugation at 8,000 r.p.m.		Fraction sedimentable at 27,000 r.p.m. in supernatant from 8,000 r.p.m. centrifugation		
		Volume, ml.	Total N, mgm.	Total N, mgm.	Amount in 8,000 r.p.m. supernatant, per cent	Portion of sediment soluble, per cent
Sarcoma 127	39.2	105	342	39.1	11.4	63
Sarcoma 160	29 (approximately)	80	212	27.2	12.8	55
Sarcoma 436	18.2	47	159	16.6	10.4	91
Normal chicken spleen	5.5	14	53	5.1	9.6	34

TABLE IV: TITER OF COMPLEMENT FIXATION OF HIGH SPEED SEDIMENT FROM SARCOMA 16 WITH HOMOLOGOUS ANTISERA

	Rabbit antiserum					Normal rabbit serum	Chicken antiserum			Normal chicken serum
Serum No	93	94	95 *	96 *	97 *	95	184	185 *	186 *	185
Titer	100	100(200)	50(100)	50(100)	50(100)	50 = complete hemolysis	6.25 = complete hemolysis			6.25 = complete hemolysis

The serum and antigen controls gave complete hemolysis. The figures give the highest dilutions with no hemolysis or with trace of hemolysis. The figures in parentheses refer to titers with slight to strong hemolysis.

* These animals were immunized with alum-precipitated high speed sediment.

Table III indicates that a relatively large amount of material sedimentable in one hour at 27,000 r.p.m. can be obtained from sarcoma 16. This is comparable to the amount yielded by a similar procedure from normal or leukemic spleen or bone marrow and is higher than that usually found in sarcoma 13 (41). Sarcoma 16 has also a higher dry weight/wet weight ratio and a higher aerobic and anaerobic acid formation per unit wet weight than sarcoma 13; the metabolism of both tumors is highly characteristic of malignant growths (11).

One chicken (No. 184), which had received high speed sediment without alum, developed fowl paralysis (neurolymphomatosis) 2½ months after the last injection. Fowl paralysis is common in chickens of this age (28, 32).

Complement fixation.—In all tests 0.2 ml. of inactivated serum dilution was mixed with 0.2 ml. of antigen dilution containing 0.01 mgm. of nitrogen and with fresh guinea pig serum diluted to contain 2 units of complement. After 45 minutes' incubation at 37° C. and 45 minutes at room temperature, 0.2 ml. of a 5 per cent suspension of sensitized sheep erythrocytes was added to each tube and the mixture incubated at 37° C. for 30 minutes.

Sera of immunized rabbits contained complement-fixing antibodies to heavy material from sarcoma 16 (Table IV). Sera of animals injected with high speed

sediment without the addition of alum reacted at higher dilutions than those immunized with alum-precipitated heavy material.

No complement fixation was obtained between sarcoma 16 heavy material and chicken sera. These included the sera of the 3 fowls immunized with high speed sediment, the serum of 1 chicken before immunization (Table IV), and the sera of 3 chickens injected with sarcoma 16 cells. The tumor had failed to grow in one of the latter and had regressed in the 2 others (Nos. 65 and 383) at the time they were bled. No complement fixation was obtained with chicken sera (Nos. 184, 185, and 186) even at dilution 1:6.25, when only 1½ units of complement were used.

Rabbit antiserum to heavy material from sarcoma 16 fixed complement at similar titers with high speed sediment from normal chicken spleen as with the homologous antigen. A rabbit antiserum to heavy material from normal chicken spleen reacted as strongly with high speed sediment from tumor as with that from normal spleen (Table V). However, after these sera had been absorbed with normal chicken spleen cells, only a slight reaction with the homologous antigen remained. The anticomplementary activity of the absorbed sera interfered with the reaction.

These sera were absorbed 3 times for 30 minutes at 37° C. with one-third their volume of packed washed spleen cells. One absorption was carried on overnight in the ice box. Sera were absorbed undiluted, and after absorption the dilution was considered to be 1:2. The unabsorbed sera were treated with saline in the same way as the absorbed ones with spleen cells. These sera also were used for neutralization experiments.

In other complement fixation tests heavy material from spleen acted as a weaker antigen than tumor sediment, but after absorption with spleen cells the antisera to tumor or spleen also reacted better with the homologous antigen. Furth and Kabat (31) failed to differentiate by complement fixation and by precipitin tests the heavy materials from sarcoma 13 and from normal chicken spleen. However, Henle, Chambers, and Groupé (38, 39) were able to identify organ-specific antigens in high speed sediments of normal tissues and Barrett (8) differentiated after absorption heavy materials from chicken tumor and from chicken embryo.

TABLE V: COMPLEMENT FIXATION TITER OF RABBIT ANTISERA TO HEAVY MATERIAL FROM SARCOMA 16 AND NORMAL CHICKEN SPLEEN WITH HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

	Rabbit antiserum to heavy material from			
	Sarcoma No. 93		Normal spleen No. 49	
Antigen	Unabsorbed	Absorbed with spleen cells	Unabsorbed	Absorbed with spleen cells
Heavy material from				
Sarcoma	100(200)	100	800	50(100)
Normal spleen	100(200)	25(50)	800	100(200)
Control (saline)	50 = complete hemolysis	25(50)	50 = complete hemolysis	50(100)

Antigen controls gave complete hemolysis.

The figures give the highest dilutions with no or with trace of hemolysis. Those in parentheses indicate the titers with slight or strong hemolysis.

NEUTRALIZATION OF AGENT 13 BY ANTISERA TO SARCOMA 16

Technic.—A filtered crude extract from sarcoma 13 (No. 679), prepared by Kabat and Furth (43) and stored at −60° C. in many small tubes, was used as a source of agent 13 in all neutralization experiments except in experiment 5. The neutralizing power of the sera in different experiments is thus comparable.

All sera were inactivated except rabbit antisera 93 and 95 in experiment 2 and chicken serum 65 in experiment 4. The serum obtained before immunization was used as a control whenever possible.

In all neutralization tests with rabbit sera a mixture of agent 13 and serum was incubated at 37° C. for 15 minutes. In experiments 1 and 2, one volume of freshly thawed extract was added to 19 volumes of serum; in experiment 3 one part of the extract was mixed with 39 parts of serum absorbed with an equal volume of cells or with 39 parts of unabsorbed serum diluted 1:2 with saline.

In neutralization tests with chicken sera one volume of extract was mixed with 49 volumes of undiluted serum, incubated at 37° C. for 45 minutes and kept for 45 minutes at room temperature.

In all tests with either rabbit or chicken sera the agent-serum mixtures were kept overnight in the ice box and injected the next day. The subsequent dilutions of the mixtures were made with ice-cooled saline immediately before injection to minimize the deleterious effect of saline on tumor-producing agents (41, 60). The concentrations of the mixtures were

always expressed in terms of dilution of the extract from sarcoma 13.

Two-tenths of a milliliter of the different mixtures were injected into the muscles of the breasts, legs, and wings of 9 to 33 day old chickens, except in experiment 1 where only breasts and legs were injected. Each chicken received all the tested serum-agent mixtures of that experiment at the same dilution. The sites of injection were alternated for the different chickens. Thus each mixture was tested in the largest possible number of animals and the influence of individual factors on the results of experiments reduced.

The chickens were examined at weekly intervals, usually on the seventh, fourteenth, etc., day. The interval between injection and the day when a distinct tumor nodule was palpated for the first time was called the latent period of that tumor.

The chickens were allowed to die, or, in a few cases, were killed when moribund. All the surviving animals were killed after 59 to 62 days (usually 60 days). In the doubtful cases, the diagnosis of sarcoma was verified by microscopic examination.

Experimental Results

Tables VI to IX give the results of the neutralization experiments. The fractions indicate the ratio of the number of successful inoculations to the number of sites inoculated with each dilution of the serum-agent mixture. The average latent period of the tumors obtained and their average diameter at death are also recorded in the tables.

The average latent period of tumors is in itself a good index of activity, as shown by Bryan and Beard (9) for the rabbit papilloma virus. This will be discussed further in connection with the results of Table VIII. The values of average latent periods, as given in the tables, are based on the day when a tumor was discovered, although the tumor appeared during the preceding week, *i. e.*, 3.5 ± 3.5 days earlier. No correction factor was introduced in the values of the latent period as this would modify all of them similarly, and the same conclusion would be reached when comparing the activity of different mixtures. This convention requires, however, that the value of the latent period of a sarcoma discovered for the first time at autopsy be given by the day of the next

Table VI: Neutralization of Agent 13 by Rabbit Antisera to Heavy Material from Sarcomas 16 and 13

Rabbit No.	Rabbit serum incubated with sarcoma 13 extract	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated
Experiment 1 (Chickens 33 Days Old at Injection)											
Dilution of injected sarcoma 13 extract.....		1:20			1:200			1:2,000			1:20,000
93	Normal	4/6	19	27	5/12	26.2	20	0/12			0/12
93	Anti-sarcoma 16	0/6			0/12			0/12			0/12
73	Anti-sarcoma 13	0/6			0/12 *			0/12			0/12
Experiment 2 (Chickens 31 Days Old at Injection)											
Dilution of injected sarcoma 13 extract.....		1:20			1:200			1:2,000			1:20,000
93	Normal	7/8	17.7	47	6/8	29.3	37	1/8	22	85	0/7
95	Normal	7/8	19.7	43	7/8	29.3	42	1 + 1†/8	43 57 †	73 9 †	0/7
93	Anti-sarcoma 16	0/8			0/8			1†/8	57 †	7 †	0/7
95	Anti-sarcoma 16	0/8			0/8			0/8			0/7
Experiment 3 (Chickens 21 Days Old at Injection)											
Dilution of injected sarcoma 13 extract.....		1:40			1:400			1:4,000			
93	Normal, diluted 1:2	6/6	21	25	2/6	21	9	0/5			
93	Anti-sarcoma 16, diluted 1:2	0/6			0/6			0/5			
93	Anti-sarcoma 16, absorbed with chicken spleen	0/12			0/12			0/10			
49	Anti-chicken spleen, diluted 1:2	12/12	19.2	29	6/12	29.2	19	1/10	28	47	

* One lymphomatous tumor.

† Possibly metastatic tumor (see text).

inspection of the animals; it is thus assumed that the nodule found at autopsy would have been discovered by palpation at the next survey.

The average diameter of tumors at death is the arithmetical average of the three diameters of all the tumors produced by the tested mixture. Minor differences in the values of the average diameter are not necessarily related to the activity of the injected material.

After injection of strongly neutralized agent 13, the few tumors that appeared were small. The sarcomas 16 produced by serum-agent mixtures grew, as a rule, progressively. Only 2 regressed completely and therefore are not included in the tables. They occurred in experiment 4 at a site injected with chicken antiserum 185 and extract diluted 1:50, and in experiment 7a at a site of injection of chicken serum 542 and extract diluted 1:500. Ten of the twelve sites injected with the former mixture and 5 of the 6 injected with the latter showed progressive tumors.

In 3 instances a large tumor occupying one breast perforated the sternum shortly before death and protruded into the opposite breast; these secondary tumors were not included in the tables.

TABLE VII: NEUTRALIZATION TESTS OF AGENT 13 BY IMMUNE AND NORMAL CHICKEN SERA

Mixture incubated: 1 part sarcoma 13 extract + 49 parts chicken serum			Dilution of injected sarcoma 13 extract								
			1:50			1:500			1:5,000		
Chicken No.	Age of chicken when bled, days	Notes on chicken bled	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.
EXPERIMENT 4 (Chickens 12 Days Old at Injection)											
185	175	Normal	10/12	21	23	9/12	32.7	20	1/12	28	20
185	215	Injected with heavy material from sarcoma 16 *	10/12	20.3	22	4/12	28	24	2/12	31.5	18
65	285	Sarcoma 16 regressed; reinjected with sarcoma 16 cells	0/12			0/12			0/12		
EXPERIMENT 5 (Chickens 27 Days Old at Injection) †											
169	359	Normal	0/9			0/9			0/9		
184	215	Injected with heavy material from sarcoma 16	3/9	30.3	19	0/9			0/9		
184	327	Same as above. Neurolymphomatosis	0/9			0/9			0/9		
186	215	Injected with heavy material from sarcoma 16 *	7/9	30	34	1/9	35	57	0/9		
450	43	Large sarcoma 16 and metastases	6/9	29.2	21	3/9	37.3	23	1/9	14	7 ‡
500	41	Large sarcoma 16; no metastases	0/9			0/9			0/9		
EXPERIMENT 6 (Chickens 9 Days Old at Injection)											
169	359	Normal	0/6			0/12			0/6		
184	215	Injected with heavy material from sarcoma 16	0/6			0/12			0/6		
184	327	Same as above. Neurolymphomatosis	0/6			0/12			0/6		
65	285	Sarcoma 16 regressed; reinjected with sarcoma 16 cells	0/6			0/12			0/6		
461	41	Large sarcoma 16; metastases	6/6	24.2	20	7/12	29	28	1/6	28	27
500	41	Large sarcoma 16; no metastases	1/6	34	9	0/12			0/6		

* Alum-precipitated.

† A different, less active preparation of sarcoma 13 extract (No. 710) was used at similar dilutions in experiment 5. At 1:50,000 a injected sites were negative.

‡ Very slowly growing tumor; only 15 × 3 × 3 mm. on the 52nd day after injection, when the chicken was killed.

NEUTRALIZATION BY RABBIT SERA

Table VI shows that rabbit antisera to heavy material from sarcoma 16 were rich in antibodies to agent 13. The amount of antibodies was not smaller than in a rabbit antiserum to heavy material from

TABLE VIII: NEUTRALIZATION OF AGENT 13 BY SERA OF CHICKENS IN WHICH SARCOMA 16 HAS REGRESSED

Chicken No.	Age of chicken when bled, days	Age of chicken when injected with sarcoma 16 cells (and size of obtained sarcoma 16 *): First injection, days	Second injection, days	Third injection, days	Mixture incubated: 1 part sarcoma 13 extract + 49 parts chicken serum; Dilution of injected sarcoma 13 extract: 1:50 Ratio: No. tumors / No. sites inoculated	1:50 Average latent period ± standard deviation, days	1:50 Average diameter of tumors at death, mm.	1:500 Ratio: No. tumors / No. sites inoculated	1:500 Average latent period ± standard deviation, days	1:500 Average diameter of tumors at death, mm.	1:5,000 Ratio: No. tumors / No. sites inoculated	1:5,000 Average latent period ± standard deviation, days	1:5,000 Average diameter of tumors at death, mm.
EXPERIMENTS 7A AND 7B (Chickens 14 Days Old at Injection)													
541 †	41	Normal			5/6	18.2 ± 3.8	16	3/6	30.3 ± 4	26	5/6	30.8 ± 3.8	9
681 ‡	49	Normal			6/6	19.8 ± 8.2	19	5/6	35 ± 7	31	1/6	42	27
682 †	49	Normal			6/6	19.8 ± 2.8	15	3/6	23.3 ± 4	20	4/6	29.7 ± 3.5	11
542 ‡	74	Normal			6/6	19.8 ± 5.3	23	5/6	28 ± 9.9	25	3/6	35 ± 14	15
543 †	74	Normal			6/6	16.3 ± 3.6	18	3/6	21 ± 7	11	6/6	35 ± 10.8	10
588 †	49	23(+++)			1/6	28	2	0/6			2/6	31.5 ± 5	21
593 †	49	23(+++)			4/6	21 ± 5.7	14	3/6	32.7 ± 10.7	23	2/6	42 ± 9.9	9
449 ‡	57	18(++)			4/6	21 ± 5.7	22	4/6	40.2 ± 14.4	11	1/6	21	42
451 ‡	57	18(++)			1/6	21	7	3/6	30.3 ± 4	13	0/6		
541 †	74	45(+)	65(0)		4/6	21 ± 5.7	14	2/6	28 ± 9.9	16	2/6	45.5 ± 14.8	9
449 ‡	91	18(++)	62(0)	82(0)	1/6	21	6	0/6			0/6		
451 ‡	91	18(++)	62(0)	82(0)	0/6			0/6			0/6		
COMBINED DATA OF EXPERIMENT													
A. Normal chicken sera (41 to 74 days old when bled; average 57.4 days)					29/30	18.8 ± 5.0	18	19/30	28.4 ± 8.2	24	19/30	33.2 ± 8.4	12
B. Sera of chickens injected once or twice with sarcoma 16 cells (49 to 74 days old when bled; average 57.2 days)					14/30	21.5 ± 5.1	15	12/30	33.8 ± 10.7	15	7/30	37 ± 11.9	17
C. Sera of chicken injected 3 times with sarcoma 16 cells (91 days old when bled)					1/12	21	6	0/12			0/12		

STATISTICAL COMPARISON OF COMBINED DATA

I. Number of tumors

Groups A and B	$\chi^2 = 16.1$ $p < 0.01$	$\chi^2 = 2.4$ $p = 0.20$–0.30	$\chi^2 = 8.2$ $p < 0.01$
Groups B and C	$\chi^2 = 3.9$ $p = 0.05$	$\chi^2 = 4.9$ $p = 0.05$–0.02	$\chi^2 = 1.6$ $p = 0.20$

II. Latent period of tumors

Groups A and B	$d = 2.7 \pm 1.6$ $p = 0.10$	$d = 5.4 \pm 3.6$ $p = 0.1$–0.2	$d = 3.8 \pm 4.9$ $p = 0.4$–0.5

III. Variation of latent period with dilution of injected agent 13

	1:50 and 1:500	1:500 and 1:5,000
Group A (normal sera)	$d = 9.6 \pm 2.1$ $p < 0.01$	$d = 4.8 \pm 2.7$ $p = 0.08$
Group B (sera of chicken injected once or twice with sarcoma 16 cells)	$d = 12.3 \pm 3.4$ $p < 0.01$	$d = 3.2 \pm 5.5$ $p = 0.5$–0.6

* The size of the sarcomas 16 obtained in both breasts is indicated by + when the average diameter of the tumors is below 5 mm., by ++ from 5 to 10 mm., and by +++ above 10 mm.

† Experiment 7b.

‡ Experiment 7a.

sarcoma 13 (experiment 1). Experiments not given in the tables indicated that diluted antisera also neutralized agent 13. Rabbit antiserum 95 neutralized the agent in dilutions 1:3, 1:9, and 1:27 but had only a questionable neutralizing action at dilution 1:81. In this experiment 49 parts of serum dilution were incubated with 1 part of extract from sarcoma 13. The neutralizing properties were exhibited in the absence of complement, as all sera were inactivated except immune sera 93 and 95 in experiment 2, which were kept for 3 weeks in the ice box.

In one chicken of experiment 2, injected with agent diluted 1:2,000 and bearing a large tumor, small tumors were found on the day of death at 2 sites injected with immune serum 93 and normal serum 95 (marked † in the table); it is possible that these tumors were metastatic, as a nodule was also found in the uninjected wing.

Triple absorption of a rabbit antiserum to sarcoma 16 with cells of normal chicken spleen failed to remove the neutralizing antibodies, although it decreased considerably the complement-fixing antibodies. A rabbit antiserum to heavy material from normal chicken spleen that fixed complement with heavy material from sarcoma 16 at higher dilution than the homologous serum had no neutralizing activity (Table VI, experiment 3, and Table V).

It has already been shown (43) that inactivated

TABLE IX: NEUTRALIZATION OF AGENT 13 BY SERA OF CHICKENS INJECTED WITH SARCOMA 16

Mixture incubated: 1 part sarcoma 13 extract + 49 parts chicken serum			Dilution of injected sarcoma 13 extract								
			1:50			1:500			1:5,000		
Chicken No.	Age of chicken when bled, days	Age of chicken when injected with sarcoma 16 cells and result of injection,* days	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.	Ratio: No. tumors / No. sites inoculated	Average latent period, days	Average diameter of tumors at death, mm.
EXPERIMENTS 8A AND 8B (Chickens 27 Days Old at Injection)											
787 †	41	16 (progressive sarcomas and microscopic metastases)	5/6	19.6	38	3/6	32.7	28	—	—	—
592 ‡	49	23 (progressive sarcomas and large metastases)	5/6	19.6	31	3/6	30.3	17	0§/6		
576 †	93	14 (+++ regressing; late sarcoma progressive)	4/6	28	23	2/6	42	16	1/6	42	7
596 †	99	23 (+ regressing; late sarcoma regressing)	4/6	24.5	18	3/6	25.7	38	2/6	45.5	8
801 †	93	Normal	3/6	35	12	2/6	31.5	17	1/6	28	7
802 ‡	93	Normal	—	—	—	2/6	28	28	2/6	42	15
803 ‡	93	Normal	—	—	—	2/6	31.5	10	0/6		
805 ‡	41	Normal	—	—	—	0/6			1/6	28	48
595 ‡	49	23 (+++ regressing)	1/6	63	6	1/6	28	16	0/6		
595 ‡	93	23 (+++ regressing)	0/6			2/6	24.5	19	1/6	56	3
580 †	93	15 (+++ regressing); 34(0)	2/6	21	19	1/6	49	6	—	—	—
452 †	91	18 (+++ regressing); 62(0); 82(0)	0/6			0/6			—	—	—
COMBINED DATA OF EXPERIMENT											
A. Chickens with progressive sarcoma and metastases (41–49 days old when bled)			10/12	19.6	35	6/12	31.5	22	0§/6		
B. Chickens bearing "late" sarcoma (93–99 days old when bled)			8/12	26.2	21	5/12	32.2	29	3/12	44.3	8
C. Normal chickens (41–93 days old when bled)			3/6	35	12	6/24	30.3	18	4/24	35	21
D. Chickens injected once or twice with sarcoma 16 cells (49–93 days old when bled)			3/18	35	15	4/18	31.5	15	1/12	56	3
E. Chicken injected 3 times with sarcoma 16 cells (91 days old when bled)			0/6			0/6			—	—	—

* Size of obtained sarcomas as in Table VIII.
† Experiment 8a.
‡ Experiment 8b.
§ One fibrous not definitely sarcomatous nodule found after 56 days.

rabbit antisera to normal chicken spleen did not neutralize agent 13. Similar results were obtained (44) in the presence of complement. Sarcoma 13 extract was incubated with 2.45 ml. of antiserum to heavy material from chicken spleen and with 0.5 ml. of fresh guinea pig serum; 4 of 10 injections of this mixture in dilution 1:50 produced tumors, whereas 6 of 10 injections of a suspension of agent 13 in the same dilution succeeded when the guinea pig serum was replaced by saline. No tumor was obtained at 10 sites that received a mixture of antiserum to sarcoma 13 and of agent 13 diluted 1:50 nor in 10 other sites inoculated with the same mixture incubated in the presence of complement. No tumor was produced by these 4 mixtures in dilution 1:1,000 of the agent. The agent preparation used in this experiment was unfortunately of low activity. One of ten control injections of agent 13 in saline, both in dilutions 1:50 and 1:1,000, were successful.

Neutralization by Chicken Sera

Injection of alum-precipitated heavy material from sarcoma 16 failed to induce neutralizing antibodies to agent 13 in 2 chickens (Nos. 185 and 186, Table VII, experiments 4 and 5). Chicken 184 had some neutralizing antibodies after immunization with heavy material from sarcoma 16 (chicken 215 days old). The neutralizing properties of the serum became stronger when this chicken developed neurolymphomatosis at 327 days of age (experiments 5 and 6). It is not possible to state that the antibodies of the sera of chicken 184 were due to immunization, as the sera of older chickens frequently contain neutralizing antibodies to tumor agents (13, 20). The serum of the untreated chicken 169, bled at the age of 359 days, neutralized completely agent 13 (experiments 5 and 6), as did serum 65, obtained from a chicken 285 days old that had received 2 injections of sarcoma 16 cells (experiments 4 and 6).

The sera of young chickens 41 to 43 days old bearing large sarcomas 16 were also tested (Table VII, experiments 5 and 6). No neutralizing antibodies were found in the sera of 2 chickens (Nos. 450 and 461) that had been injected with sarcoma 16 cells at the ages of 18 and 4 days, respectively; when bled to death both chickens had tumors about $60 \times 30 \times 30$ mm. in the breasts, sarcoma nodules in the peritoneal cavity, and metastases in the lungs. Chicken 500 was injected with sarcoma 16 cells at the age of 13 days and when bled to death had large tumors in the breasts but no tumor elsewhere; its serum neutralized agent 13. These results suggested that the resistance to the growth of sarcoma 16 could be correlated with the presence of antibodies to agent 13.

The sera of chickens injected 1 to 3 times with sarcoma 16 cells in which the tumors had regressed, were tested and compared with the sera of normal chickens of the same age and of chickens with progressive tumors (Tables VIII and IX).

The twelve sera-agent mixtures of Table VIII were made with the same preparation of agent 13 and injected the next day in 2 groups of 18 chickens, each of which received 6 of the 12 mixtures at the same dilution (experiments 7a and 7b, respectively). In the same way, the 12 sera of Table VIII were tested simultaneously in 2 groups of 15 chickens (experiments 8a and 8b).

Table VIII shows that only the sera of 2 chickens, 91 days old, injected previously 3 times with sarcoma 16 cells, neutralized almost completely agent 13. A smaller degree of neutralization was produced by the sera obtained from these 2 chickens after only 1 injection of sarcoma 16 cells, as well as by the sera of 3 other chickens, 49 and 74 days old, injected once or twice with sarcoma 16 cells. These 5 sera have definite ability to neutralize agent 13 if they are compared with the sera of 5 normal chickens of similar age.

At each dilution of the agent the grouped results show a larger number of successful inoculations and a shorter average latent period for the normal sera than for the sera of chickens injected once or twice with sarcoma 16. The χ^2 test, applied to the number of tumors and calculated according to Yates' corrected formula for small numbers (40, page 93), shows significant differences at dilutions 1:50 and 1:5,000; at dilution 1:500 the difference is not significant but the whole experiment is significant ($\chi^2 = 16.1 + 2.4 + 8.2 = 26.7$); the probability of occurrence by chance of a difference in the number of tumors as large as or larger than observed is smaller than 0.01. The differences of the average latent periods of the tumors of the 2 groups at dilutions 1:50 and 1:500 are not significant when their standard errors are considered. For the determination of the standard deviation $n-1$ was used; the value of the probability was derived from that of t in Fisher's table (23). The trend indicated by the larger values of the latent periods agrees, however, with the smaller number of tumors obtained when the agent was incubated with antisera to sarcoma 16.

The large number of preparations of agent 13 tested in identical conditions in experiments 7a and 7b (Table VIII) enables a consideration of the relation between *latent period and dilution of agent 13*. There is a highly significant increase of average latent periods of tumors between dilutions 1:50 and 1:500 of the agent, after incubation with both normal and immune sera. In the range 1:500 to 1:5,000 the average latent periods still increase, but the differences are smaller

and less than twice their standard errors. A linear relation between the average latent periods of tumors and the logarithm of the amount of injected agent has been demonstrated for carcinogenic chemicals (10, 33) and for the rabbit papilloma virus (9). A similar relationship may exist for amounts of agent 13 corresponding to a 1:20 to 1:500 dilution of preparation 679.

Fig. 5 illustrates the relation between the average latent period of the tumors obtained in each experiment with the nonneutralizing sera and the logarithm of the dilution of the preparation of agent 13 that

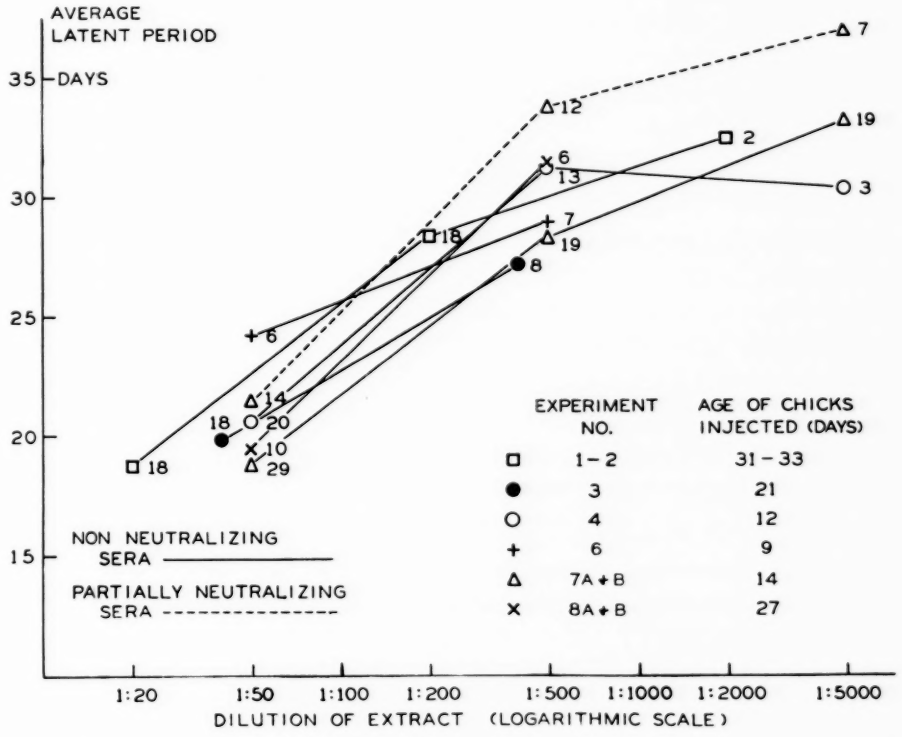

FIG. 5.—Relationship between the average latent period of tumors and the amount of agent 13 injected.

An extract from sarcoma 13 (preparation 679) was incubated in dilutions 1:20 to 1:50 with serum; further dilutions were made with saline. The values of average latent periods, given as solid lines, were based on the combined data obtained with nonneutralizing sera. Values obtained in experiments 7a and 7b with partially neutralizing sera are shown by the broken lines.

The figures on the graph indicate the number of tumors on which the averages are based.

was used. The different lines are closely grouped and fairly parallel in the range 1:20 to 1:500. At higher dilution, however, their slope becomes less and they are more scattered; in one instance the average latent period even decreases slightly between dilution 1:500 and 1:5,000. This scattering is readily explained by the smaller number of tumors on which the averages at higher dilutions are based and by the larger variability of the individual values. A similar increase in the standard deviation of latent periods of tumors induced by higher dilutions of carcinogenic compounds has been noted by Dunning, Curtis, and Wood (18), and Bryan and Shimkin (10).

The constant flattening of the lines of Fig. 5, as well as the comparison of the differences in the latent period recorded in Table VIII, indicate, however, that another factor influences the results at higher dilutions. It is very unlikely that the animals have been killed too early and that many tumors have thus been missed. When the surviving animals of experiments 7a and 7b were killed on the 61st day, 3 times the value of the standard deviation had elapsed after the average latent period of one group of tumors produced by agent diluted 1:5,000 and 2 times that value for the group that had received antisera to sarcoma 16 and agent diluted 1:5,000. It is more probable that, in animals injected with several mixtures containing a high dilution of the agent, the first tumors kill the chicken before the tumors with longer latent periods have time to develop; this is less important with a more active suspension of the agent, as there is less variability in the latent periods. However, in animals that received only one active mixture in high dilution, the number of induced tumors is not proportionally larger (experiments 1 and 6). It is probable that a general immunity to the injected materials develops in the chickens after 1 month; this is indicated by the regression of a few tumors and it would explain the scarcity of tumors appearing later. A similar phenomenon concerning

the occurrence of papillomas in rabbits has been discussed by Bryan and Beard (9).

The paradoxical increase of the number of tumors with dilution, observed in a few cases, is probably due to the variability inherent in tests with small numbers and not to the "dilution phenomenon," dissociation of the virus-antibody complex, described by Todd for fowl plague virus (69) and by Andrewes for vaccine virus (3). Andrewes could not show any dissociation of the Rous agent-antibody complex after a contact of more than 10 minutes at room temperature (5).

It is concluded that low values of latent periods, under 30 days, obtained under conditions of the present experiments can be used as a measure of the relative activity of the material injected; longer average latent periods indicate a lower activity but cannot be so readily accepted as indices of minor differences, inasmuch as they are based on small numbers of tumors.

The results of Table VIII do not indicate whether the strong neutralizing activity of sera of chickens injected 3 times with sarcoma 16 cells was due to their age, 91 days, or to repeated injections. That the latter interpretation is correct is shown by the results recorded in Table IX.

The serum of chicken 452, 91 days old, that similarly had been injected 3 times with sarcoma 16 cells completely neutralized agent 13. On the 35th day a tumor was found in the subcutaneous tissue of the left mesotarsal joint of a chicken that had been injected in the corresponding thigh with serum 452 and agent 13 diluted 1:500; microscopically this tumor proved to be a spindle cell sarcoma. The chicken developed no other tumor. The injection from which this sarcoma originated cannot be ascertained.

The sera of 3 normal chickens 93 days of age (Nos. 801, 802, and 803) and one 41 days old (No. 805) neutralized agent 13 only slightly. The sera of 2 birds (Nos. 595 and 580) that had been injected once or twice with sarcoma 16 cells contained more antibodies to agent 13 than the sera of normal chickens of the same age and less than the serum of chicken 452 that had received 3 grafts of sarcoma 16. The amount of antibodies did not increase in chicken 595 from the time sarcoma 16 was still regressing, 49 days old, until the tumor had completely regressed, 93 days old.

The differences between the 3 groups of chickens considered, C, D, and E, in Table IX, are not large enough to be significant by the χ^2 method for small numbers. It seems likely, however, that the neutralizing power of the sera increases from groups A to E in Table IX: the percentage of positive inoculations becomes less at dilutions 1:50 and 1:500 as the average latent period at dilution 1:50 increases.

The sera of 2 chickens with a late sarcoma 16 (Nos. 576 and 596) contained less neutralizing antibodies to agent 13 than the sera of chickens of the same age that had been grafted once or twice with sarcoma 16 cells and in which the tumor had vanished. The difference in the number of induced tumors is significant (at dilution 1:50: $\chi^2=5.7$; $p=0.02$). The sera of birds bearing late tumors contained no more antibodies, and perhaps less, than the sera of uninjected chickens of the same age. The failure of the injection of sarcoma 16 cells to produce a lasting immunity in these animals can be correlated with the recurrence of the late sarcomas.

The sera of 2 young chickens (Nos. 592 and 787) having large sarcomas 16 with metastases failed to neutralize agent 13. They differed significantly from the sera of chickens with tumors that regressed (at dilution 1:50: $\chi^2=10.5$; $p<0.01$).

Chicken 592 had huge tumors, $70\times25\times25$ mm., in the breasts and large metastases in the liver, peritoneum, mediastinum, and lungs. Chicken 787 had large tumors in the breasts, $50\times25\times15$ mm. (Figs. 1 and 2); although there were no gross metastases, sections of the lung, liver, and kidney showed numerous intravascular sarcomatous foci with incipient parenchymatous infiltration (Figs. 3 and 4). Two chickens (Nos. 450 and 461, Table VII) that had large sarcomas 16 and metastases, also lacked neutralizing antibodies to agent 13, whereas chicken 500 with large tumors and no metastasis had strong antibodies in its serum.

It has thus been shown that the resistance to the growth of sarcoma 16 in chickens is associated with the presence in their sera of antibodies effective against agent 13.

RESISTANCE OF CHICKENS INJECTED WITH SARCOMA 16 CELLS TO A SUBSEQUENT INJECTION OF AGENT 13

Technic.—Dilutions in saline of an extract from sarcoma 13 (No. 679) were injected into chickens in which sarcoma 16 had regressed and into normal chickens of the same age. The sera of many of these birds had been tested in the neutralization experiments. Two-tenths of a milliliter of the extract diluted 1:50 was injected into the right wing, breast, and leg and 0.2 ml. diluted 1:500 into the left wing, breast, and leg. Table X includes the results of injections made on 3 different days (experiments 7c, 7d, and 8c, respectively). The same sample of extract was used in experiments 8a, 8b, and 8c. The surviving chickens were killed after 60 or 61 days.

The resistance to injections of agent 13 increases slowly with age in normal chickens. Adults are also more resistant than young chickens to the leukosis agent 1 (47) and to the Rous sarcoma agent (13, 20).

TABLE X: RESISTANCE TO AGENT 13 OF CHICKENS IN WHICH SARCOMA 16 HAS REGRESSED

Chicken No.	Age of chicken at time of injection with: Sarcoma 16 cells (and size of sarcoma obtained *) First injection, days	Second injection, days	Third injection, days	Agent 13, days	Dilution of injected sarcoma 13 extract: 1:50 Ratio: No. tumors / No. sites inoculated	1:50 Average latent period ± standard deviation, days	1:50 Average diameter of tumors at death, mm.	1:500 Ratio: No. tumors / No. sites inoculated	1:500 Average latent period ± standard deviation, days	1:500 Average diameter of tumors at death, mm.	Neutralizing activity of the serum: Degree	Age of chicken when bled, days
A. CONTROLS												
805 †				47	3/3	19.7 ± 4	27	2/3	32.5 ± 4.9	10	Strong	41
806 †				47	3/3	33.7 ± 16.2	6	0/3			—	—
681 ‡				49	3/3	28 ± 0	26	2/3	28 ± 0	9	0	49
781 §				65	3/3	25.3 ± 6.5	31	0/3			—	—
782 §				65	2/3	28.5 ± 4.9	25	1/3	39	3	—	—
543 ‡				80	3/3	42 ± 12.1	17	0/3			0	74
801 †				100	0/3			0/3			Moderate	93
802 †				100	3/3	33 ± 15.1	37	1/3	54	3	Moderate	83
803 †				100	2/3	42 ± 0	33	0/3			Moderate	93
169 †				450	0/3			0/3			Complete	359
B. INJECTED ONCE WITH SARCOMA 16												
593 ‡	23 (+++)			49	0/3			0/3			Moderate	49
589 †	23 (+++)			100	0/3			0/3			—	—
595 †	23 (+++)			100	1‖/3	63	4	0/3			Strong	93
597 †	23 (++)			100	0/3			0/3			—	—
672 †	44 (±)			100	0/3			0/3			—	—
673 †	44 (+)			100	0/3			0/3			—	—
C. INJECTED TWICE WITH SARCOMA 16												
541 ‡	45 (+)	65 (0)		80	0/3			0/3			Moderate	74
580 †	15 (+++)	34 (0)		100	0/3			0/3			Strong	93
582 †	15 (++)	34 (±)		100	0/3			0/3			—	—
531 †	13 (+++)	48 (0)		114	0/3			0/3			—	—
65 †	117 (+)	169 (0)		450	0/3			0/3			Complete	285
D. INJECTED THREE TIMES WITH SARCOMA 16												
449 ‡	18 (++)	62 (0)	82 (0)	97	0/3			0/3			Almost complete	91
452 ‡	18 (+++)	62 (0)	82 (0)	97	1‖/3	21	8	0/3			Complete	91
451 §	18 (++)	62 (0)	82 (0)	113	0/3			0/3			Complete	91
COMBINED DATA												
											Number of chickens susceptible to agent 13	
Controls				47– 65	14/15	26.9 ± 8.7	23	5/15	32 ± 5.1	8	5/5	
Controls				80–100	8/12	38.6 ± 11.4	29	1/12	54	3	3/4	
Controls				450	0/3			0/3			0/1	
Previously injected with sarcoma 16				49	0/3			0/3			0/1	
Previously injected with sarcoma 16				80–114	2‖/36	42 ± 29.7	6	0/36			2?/12	
Previously injected with sarcoma 16				450	0/3			0/3			0/1	

* Size of sarcoma 16 obtained as in Table VIII.
† Experiment 8c.
‡ Experiment 7c.
§ Experiment 7d.
‖ Fibrotic sarcoma nodule.

A larger proportion of injections of agent 13 was successful in chickens 47 to 65 days old than in chickens 80 to 100 days old; only 1 of 4 of the latter developed a tumor at dilution 1:500 of the agent, whereas 3 of 5 animals of the young group were susceptible to that dilution. No tumor was obtained in an untreated fowl 450 days of age.

There was a parallel increase of the latent period of tumors induced in older birds by agent 13. The chicks 9 to 33 days old used in the neutralization experiments developed tumors after about 20 days following the injection of extract diluted 1:50 in normal sera (Fig. 5). Chickens 47 to 65 days old injected with extract diluted 1:50 in saline had tumors after 26.9 days (Table X). The average latent period was larger in normal chickens 80 to 100 days of age injected with agent 13 diluted 1:50 in saline. This difference is significant ($d = 11.7 \pm 4.6$; $p = 0.02$).

Chickens previously injected with sarcoma 16 cells showed a much higher resistance to agent 13 than control animals of the same age. Whereas 8 of 12 injections of the agent diluted 1:50 were successful in control chickens 80 to 100 days old, only 2 of 36 succeeded in chickens of similar ages in which sarcoma 16 had regressed; furthermore, the latter 2 tumors were small fibrosarcomas of very low malignancy. The number of previous injections (1 to 3) with sarcoma 16 cells seems unimportant in determining the degree of resistance to agent 13.

When groups of chickens are considered, there is a parallel in the increase of resistance to agent 13 and the amount of antibodies in the sera (Table X). Chickens without antibodies in their sera were susceptible to agent 13, and most of the chickens whose sera completely neutralized agent 13 failed to develop tumors. However, there are individual variations, and moderate to strong neutralizing power of the sera was as compatible with susceptibility as with resistance of the chicken to agent 13. Duran-Reynals (20) showed some parallelism between resistance of old chickens to the Rous agent and the amount of circulating antibodies. Discrepancies between the immunity of animals and the amount of neutralizing antibodies larger than those shown in Table X have been observed after vaccination with the viruses of St. Louis encephalitis, rabies, and poliomyelitis, and after recovery from poliomyelitis (see Rivers, 58). The results in Table X indicate that the general resistance of chickens to agent 13 is conditioned by factors similar to those determining the production of circulating antibodies. Nevertheless, the neutralizing antibodies are not the only determinant of resistance to viruses or tumor agents.

DISCUSSION

In the present experiments, antibodies appearing in response to the injection of cells or heavy material from a nonfilterable chicken tumor (sarcoma 16) neutralized a filterable agent of leukosis and sarcoma (agent 13).

These neutralizing antibodies acted directly, *in vivo* or *in vitro,* upon agent 13. It is indeed unlikely that the antibodies were directed against a constituent of tumor cells common to sarcomas 13 and 16 and different from the antigen of agent 13. The sera were probably completely resorbed from the injected site or destroyed at the time the first cells of sarcoma 13 appeared. Furthermore, no difference was found in the results of the neutralization experiment with dilutions 1:50 and 1:5,000 of the agent. With the latter dilution, smaller amounts of antisera were injected and the tumor cells appeared after a longer latent period. The development of a defense mechanism against the growth of any tumor cells, and not against agent 13, could, however, explain the resistance acquired by chickens to agent 13 after regression of sarcoma 16 (Table X), but this explanation cannot be extended to the results of the neutralization experiments (Tables VI to IX).

The neutralizing antibodies were not developed against a normal fowl constituent associated with agent 13. Neutralization of total extracts of Rous sarcoma 1 by antisera to normal fowl tissues has been reported only in the presence of complement; absorption of these sera with normal chicken tissues removed the neutralizing antibodies (1, 2, 35). The antisera to filterable (8, 43, 60) and nonfilterable tumors (25, 26) obtained by other investigators neutralized tumor agents even in the absence of complement (25, 26, 43, 60) and after absorption with normal fowl tissue (8, 25, 26, 43, 60). Likewise, the rabbit antisera to the nonfilterable sarcoma 16 neutralized agent 13 in the absence of complement and after absorption with normal fowl tissue, whereas rabbit antisera to normal fowl tissue had no neutralizing properties.

In chickens it is unlikely that the antibodies developed by the injection of sarcoma 16 cells were directed against a normal constituent of fowl tissue. These chicken antibodies did not fix complement with heavy material from sarcoma 16 and differ from the natural antibodies recently found in rabbit sera by Kidd and Friedewald (45, 46), which fix complement with a sedimentable constituent of normal cells. Thus the neutralizing antibodies developed by sarcoma 16, like those obtained with crude and purified preparations of Rous agent by Murphy, Sturm, Favilli, Hoffman, and Claude (54), are unrelated to the complement-fixing antibodies "developed against the incidental proteins of the tumor."

As the antibodies developed by sarcoma 16 acted directly upon agent 13, it must be concluded that the nonfilterable sarcoma 16 contains an antigen closely

related to that of the leukosis sarcoma agent 13. Several explanations can be given:—

1. Sarcoma 16 cells are associated accidentally with a filterable agent or a virus different from agent 13, but containing a related antigen. Cross reactions between the agents of several chicken tumors have been described (4, 5, 6); agent 11 is partly neutralized by antisera to agent 13 (43). The virus of lymphogranuloma inguinale when injected into mice is known to localize in spontaneous (61) and grafted tumors; it is transmitted with them, but the grafted mice show lesions of the disease and extracts of the tumors reproduce lymphogranuloma inguinale (62). Rivers and Pearce (57) showed that vaccine and virus III multiply in a rabbit tumor and are carried along through an indefinite number of transplants; the viruses are demonstrable in the tumors despite the immunity developed by the rabbit host. Mellanby (52) easily recovered the Rous agent from a chemically induced tumor borne by a chicken that had been injected at a different site with Rous agent.

No other disease was regularly found in chickens injected with sarcoma 16 cells. The incidence of neurovisceral lymphomatosis was not higher among them than among normal chickens (28, 32): only 3 cases of that disease were found among all chickens injected with sarcoma 16 material. A contamination of sarcoma 16 cells by agent 1 has to be considered, since the chicken in which sarcoma 16 originated was also injected with agent 1. Erythroleukosis, the disease produced by agent 1, however, was never observed in animals treated with cells, filtrate, lyophilized tissue, or heavy sediment from sarcoma 16. This is in contrast to the experience of Mellanby (52), Schoen (61), and Rivers and Pearce (57), who easily recovered viruses from contaminated tumors. Furth (29) was also able to re-isolate the agent of leukosis 1 and the agent of sarcoma 11 after injection into the same animal. It is most likely that agent 1 died out after having been injected into the original chicken bearing sarcoma 16, as no direct evidence of its presence was ever obtained. However, the present findings point to the difficulties in distinguishing between the presence of normal tissue antigens and that of a latent symbiotic virus carried by animals used in transfers. Older chickens spontaneously develop antibodies to agent 13, and also to tissue antigens (19), to vaccine virus (21), and to several neoplastic agents (20) with which they apparently had no contact. If a contamination of sarcoma 16 by agent 1 or by a latent virus is invoked to explain the present results, a similar latent infection must also be assumed to explain the results of other investigators working independently with chemically induced tumors (7, 17, 25, 26).

2. Sarcoma 16 is induced by and necessarily associated with a filterable agent. Filterable sarcomas of fowl are known to undergo phases of nonfilterability (34). However, all attempts to demonstrate a filterable agent in sarcoma 16 thus far have failed. These included the method suggested by Duran-Reynals (22), namely, passage through ducklings. Agent 13 has been recovered from similar heterologous transfers made with sarcoma 13. Most authors agree on the nonfilterability of chemically induced sarcomas of fowls (25, 37, 51, 53, 55, 56, 59, 68).

3. The cells of sarcoma 16 contain a specific antigen that is unable to initiate a malignant process in a normal cell and that resembles closely constituents of agent 13 or is similar to it. Rivers (58) considers that the Rous agent, unlike the large viruses, contains only one antigen. Leukosis agent 1, and presumably its variant, agent 13, are about the same size as the Rous agent, approximately 70 mμ (65, 66). According to Foulds and Dmochowski (26), however, the Rous sarcoma and a chemically induced chicken tumor contain two antigens not demonstrated in normal fowl tissue, of which one passes membranes that retain the Rous agent. The presence of normal fowl constituents in tumor agents (1, 2, 35, 48) is not generally admitted (7, 24, 25, 26). In recent studies with influenza virus, Chambers, Henle, Lauffer, and Anderson (14, 15) suggest that the infectious units of filterable tumors may be smaller than hitherto believed and adsorbed on tissue components. Kabat and Furth (41) have shown that the bulk of the high speed sediment of sarcoma 13 is similar to that of normal tissue. Whatever the size of tumor agents, the antibodies induced by sarcoma 16 are directed against agent 13 and not against normal fowl components included in or associated with it.

These experiments indicate the presence in a chemically induced chicken sarcoma of a specific antigen also found in the filterable agent 13 of leukosis and sarcoma. This antigen appears to be associated with the activity of tumor cells. It is possible that the specific antigen is contained in a tumor agent that loses its infectivity when freed from cells, even though it retains antigenicity. Antibodies may be instrumental in this inactivation. It has been shown that antibodies interfere with the growth and the spread of sarcoma 16 cells and also neutralize the filterable agent 13. The nature of the association of the specific antigen with tumor cells may also preclude the isolation from the cells of an active tumor agent. The stability of this association or the alteration of the antigen when it is isolated probably differentiates the nonfilterable from the filterable chicken tumors.

SUMMARY

The nonfilterable chicken sarcoma 16 originally induced by methylcholanthrene was transmitted for 27

generations in chickens. In young chicks the tumor was invasive and often metastasized, but it frequently regressed in older birds. All attempts to isolate a filterable agent from this tumor failed. Sarcoma 16 grew readily in newly hatched ducklings but no filterable agent was found in the duck-grown tumor.

The regression of sarcoma 16 rendered chickens resistant to a subsequent injection of a filterable agent of leukosis and sarcoma (agent 13). Their sera contained neutralizing antibodies to this agent in much larger amounts than those of normal birds of the same age. The sera of chickens in which sarcoma 16 was growing but did not metastasize also strongly neutralized agent 13. In the sera of chickens with metastases no neutralizing antibodies were found. Sarcoma 16 appeared or recurred in a few chickens several months after the graft of particles; these birds had fewer neutralizing antibodies to agent 13 than chickens that had resisted the injection.

Neutralizing antibodies to agent 13 were also produced by injecting rabbits with high speed sediments from sarcoma 16. These antibodies could not be absorbed by cells from normal chicken spleen, which decreased considerably the complement-fixing antibodies. The injection into rabbits of heavy material from normal chicken spleen did not produce neutralizing antibodies to agent 13.

Thus a nonfilterable chemically induced chicken sarcoma contains an antigen not found in normal fowl cells. This antigen is related to an antigen contained in a filterable agent of leukosis and sarcoma (agent 13).

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On the Quantitative Evaluation of Experimental Skin Carcinogenesis by Methylcholanthrene*

The Factors of Dosage, Time, Spacing of Applications, and the Multiplicity of the Carcinogenic Response

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INTRODUCTION

Development of the Technic of Experimental Carcinogenesis

Although the process of experimental carcinogenesis as studied by the external application to the surface of the skin of solutions of chemical carcinogens has been the subject of numerous investigations, the factor of dosage has received little attention so far. The term dosage means the quantitative evaluation of the amount of a chemical carcinogen delivered to a skin area of a definite size. In the present paper we have gathered together the results of a series of experiments, in which the dosage of the carcinogen and the spacing in time of its application have been varied. Some of these experiments are described for the first time in the first part of this paper, others have been published already. A comparison of the results of these various experimental series indicates that the importance of the time factor has been unduly exaggerated, owing, as we believe, to an incomplete conception of the way in which chemical carcinogens act. This conception was formed in the early days of experimental carcinogenesis when only relatively weak chemical carcinogens such as coal tar were available.

The technic of inducing cancer in the skin of mice by chemical carcinogens and of measuring the activity of the various carcinogens has remained unchanged since that period. In those days cancer was induced by painting the skin with chloroform or benzene solutions of tar—either tar as such or tar from which water-soluble toxic substances, which were not carcinogenic, had been removed by various methods. The applications were made 2 or 3 times weekly. It was believed at that time that cancer could be made to develop only if the normal tissue was continuously exposed to the chemical carcinogen. This belief was mainly based on the fact that if the applications of tar were made at longer intervals, from 4 to 6 days, the carcinogenic effect diminished progressively. In an experiment (3) in which applications of a tar preparation were made at intervals of 7 days for 6 months to a small skin area no carcinomas developed, while similar applications of the same tar twice weekly for 3 months elicited skin cancer in a considerable percentage of the animals. In this experiment the total number of applications and therefore the dose of the carcinogen was the same, so that the different response had to be attributed to the different time intervals at which the tar was applied.

The application of tar at intervals of 2 or 3 days was assumed to ensure a continuous exposure of the skin to the carcinogen. This assumption has since been proved to be correct by later observations with ultraviolet light, which elicits a striking fluorescence in many substances carcinogenic for the skin. They disappear from the surface of the skin and have been stated to be completely absent after intervals varying from 6 days to 2 weeks as judged by naked eye inspection. Recent microscopic observations by Simpson and Cramer (13) on frozen sections of mouse skin to which one application of methylcholanthrene in benzene had been made have confirmed these findings. The emphasis laid on the importance of a continuous exposure of the skin to the carcinogen was retained when tar, which contained only a small percentage of the chemical carcinogen benzpyrene, was replaced by pure organic substances that were potent carcinogens. This gave rise to the concept that chemical carcinogens owe their effect to a direct stimulating action on the epithelial cells of the skin. These substances have, in fact, been classed by some biologists among the group of growth-promoting substances (11).

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The use of pure chemical carcinogens that were more potent than tar and could be applied to the skin in higher concentrations shortened greatly the latent period. It also increased the percentage of animals in which skin cancer could be elicited, and with such potent carcinogens as benzpyrene or methylcholanthrene cancer could be made to develop in 100 per cent of the animals. As a measure of the relative effectiveness of the various carcinogenic hydrocarbons, the ratio of the percentage of cancerous mice to the average length of the latent period has been used as a convenient index. When cancer is induced in 100 per cent of the mice the length of the latent period thus becomes a reciprocal measure of the carcinogenic activity of a substance. Fieser is of the opinion that for all chemical carcinogens "the rate of tumor formation provides the best index available for estimating the relative activities" (9).

This brief historical account has been given in order to set out the factual basis for the conception of a direct growth-stimulating action of chemical carcinogens on epithelial cells maintained over a long period of time, as an explanation of their carcinogenic power. It also accounts for the emphasis laid on the time factor as a measure of carcinogenic activity in disregard of the factor of dosage, the yardstick usually employed in the study of the biological activity of chemical substances.

In our systematic study of the early stages of skin carcinogenesis by methylcholanthrene (6, 7) it was found that the first single application of methylcholanthrene to the skin produces immediately a damage to the epithelial cells of the epidermis and its appendages, *i.e.* hair follicles and sebaceous glands. The hairs fall out and the sebaceous glands disappear completely. This is followed by a process of regeneration, which is completed rapidly in the epidermal epithelium but very slowly in the sebaceous glands. This biological effect is accompanied by an immediate change in the chemical composition of the epidermis that persists for many weeks after a single application (1, 15).

An area of skin that has been exposed to a single application of methylcholanthrene undergoes, therefore, a profound change both in its structure and in its chemical composition, so that for a while the cells of that area live in a changed environment. During this period both the epidermis and the hair follicles undergo in a considerable fraction of the treated animals a proliferation, which is progressive over a period of several weeks to form a massive hyperplasia, histologically closely resembling conditions known clinically in man as precancerous. In some animals this may even proceed to the development of carcinoma after an interval varying from 4 to 9 months (8).

Naked eye and microscopic observations already referred to agree that the chemical carcinogen does not persist in the skin as such for more than 2 weeks at the most after a single application to the skin. These facts are not readily compatible with the conception of a direct stimulating effect of the carcinogen on the epithelial cells. They suggest rather that the progressive epithelial proliferation is an indirect effect that sets in as a reaction to the damage produced by the carcinogen and that may conceivably be due to a new chemical substance formed in the skin. If this were so, the continuous exposure of the skin to a carcinogen would not be as essential a condition for carcinogenesis as it has been held to be, and a more rational method of application would be one in which the epithelial proliferation is allowed to run its course before a second application of the carcinogen is made, thus allowing a longer interval of time to elapse between successive applications.

EXPERIMENTAL

In order to test the validity of such a conception a number of experiments have been carried out. In these the routine technic of a *continuous exposure* to the carcinogen, which involves frequent applications at short intervals, and therefore a massive dosage of the carcinogen, was abandoned for a *discontinuous exposure,* in which the carcinogen was applied at increasing intervals of time and the dosage could be greatly reduced. Preliminary experiments, the results of which have been published (5), gave support to the view outlined above by showing that the number of applications, *i.e.* the dosage necessary to induce cancer in 100 per cent of the animals, diminishes as the time interval between successive applications is increased. These experiments have now been repeated on larger series of animals. This paper records the results obtained, together with other observations designed to explore the effect of reducing the total dose of carcinogen applied to the skin. This has been effected in two ways: (a) by varying the technic of a continuous exposure to a technic of discontinuous exposure as explained above, (b) by reducing the number of applications and limiting them to the initial stage of the experiment.

The experimental conditions observed in these experiments are the same as those used and described in previous communications, unless specifically stated to be otherwise. They are: Female Swiss mice from 2 to 3 months old supplied by Tumblebrook Farm; a 0.6 per cent solution of 20-methylcholanthrene in benzene applied by a No. 4 brush to the middle line of the back with a single stroke in an axial direction from the nape of the neck to the middle of the back, any excess of the benzene solution adhering having been removed by tapping the brush on the mouth of

the glass vessel. The amount of methylcholanthrene applied to the back in this manner at each brush stroke was found to be approximately 0.1 mgm. (5).

The animals were examined once weekly for the presence of a malignant neoplasm and the time when malignancy was first diagnosed clinically was recorded as described in a previous communication. When the mice were killed a sketch was made to show the presence of all the tumors, benign and malignant, that had developed. The whole affected skin area was fixed and for microscopic examination pieces of the skin were removed sufficient in size and number to include all the malignant tumors present. In some animals as many as 4 pieces were removed for examination.

100 Per Cent Skin Carcinogenesis by the Method of Discontinuous Exposure of the Skin

The following experiments were carried out in order to confirm on a larger number of animals the results obtained in our preliminary observations on the effects of the protracted method of applying the carcinogen; *i.e.*, the application at long intervals of time. Three experimental series of mice were laid down:

Series C. 43 mice. Application made once every 2 weeks.
Series D. 44 mice. Application made once every 3 weeks.
Series E. 48 mice. Application made once every 4 weeks.

threne in 0.1 mgm., each 0.1 mgm. representing an application of the carcinogen. In both figures the ordinates represent the percentage of cancerous animals calculated on the number of effective mice; *i.e.*,

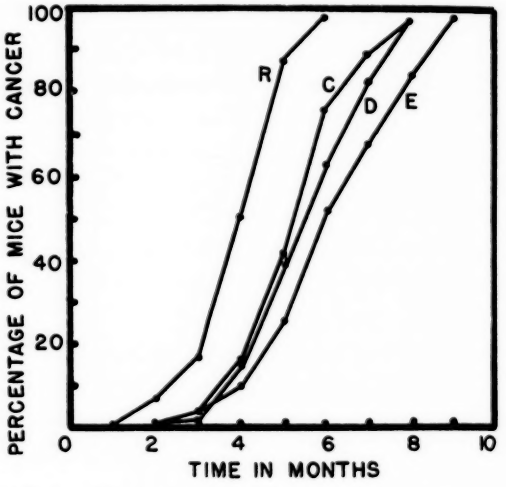


Fig. 1.—Incidence in mice of skin cancer, relative to time, following discontinuous exposure to methylcholanthrene at intervals of 2 (C), 3 (D), and 4 weeks (E). Graph R represents the cancer incidence following continuous exposure as practiced in the routine technic; *i.e.*, 0.6 per cent methylcholanthrene applied 3 times weekly for 14 weeks.

mice alive when the first malignant tumor appeared. The reason the curves do not reach the 100 per cent line is that in each experimental group 1 or 2 mice died before the experiment was completed. But in all

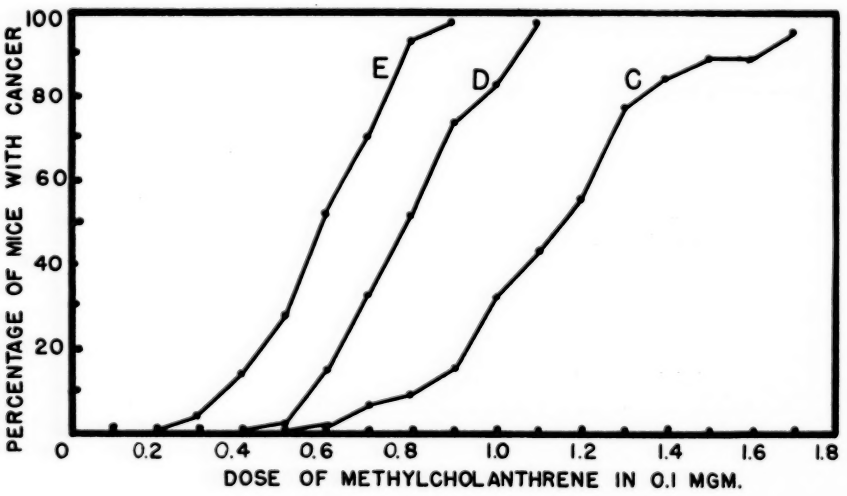


Fig. 2.—Incidence in mice of skin cancer, relative to dosage of methylcholanthrene, following discontinuous exposure to methylcholanthrene at intervals of 2 (C), 3 (D) and 4 weeks (E). Compare with same experiment in Fig. 1.

The applications were continued, with observation of the various time intervals given above, until all the surviving mice had developed cancer. The results are recorded in relation to time in Fig. 1 and in relation to the dosage of the carcinogen in Fig. 2, where the abscissa indicates the amounts of methylcholanthree series skin cancer was present at the termination of the experiment in all the surviving mice. When the process of carcinogenesis in response to the various types of protracted applications of the carcinogen is recorded in the usual way in relation to time, as in Fig. 1, the graphs for series C and D run closely

together throughout the experimental period, and this applies also to series E for the early period until about 20 per cent of the mice have developed cancer. Subsequently series E lags slightly behind the other two series, C and D. It is surprising to find that the differences in dosage, which is twice as high in series C as in series E, are not reflected in correspondingly large differences in the carcinogenic response when measured in terms of time.

Evaluation of the Carcinogenic Response by Time or by Dosage

Striking differences appear when the carcinogenic response is measured in terms of dosage (Fig. 2). It is then seen that in agreement with our earlier observations the total dose necessary to induce cancer in 100 per cent of the animals diminishes as the exposure of the skin to the carcinogen becomes increasingly discontinuous. Table I shows those dosages together with the dosage required in the routine technic of a continuous exposure when the same concentration of methylcholanthrene in benzene is applied (0.6 per cent) 3 times a week for 14 weeks. The carcinogenic response to this routine technic in relation to time is recorded in Fig. 1 as graph R.

It is evident that the evaluation of the process of carcinogenesis by time is defective in so far as it fails to reveal differences that are disclosed when the process is measured in terms of dosage.

A similar failure to detect a relation between the carcinogenic response and dosage is found when the continuous exposure method of carcinogenesis by frequent applications at short intervals is used. For that purpose the effects of a 0.6 per cent solution of methylcholanthrene in benzene (graph R) was compared with those of a 0.3 per cent (graph B) and of a 1 per cent solution (graph A), all other experimental conditions such as frequency of applications, size of area, etc., remaining the same. Since the dose of methylcholanthrene administered to a large skin area by one brush stroke of a 0.6 per cent solution is 0.1 mgm., the amount applied when the 0.3 per cent solution is used can be assessed at about 0.05 mgm. for each brush stroke and for the 1 per cent solution at about 0.15 mgm. The results, as represented in Fig. 3, with the abscissa indicating the length of the latent periods in months, show in the early stages of the process of carcinogenesis a close similarity in spite of the great differences in the dosage. The appearance of the first carcinomas requires the same period of induction of about 2 months for the 0.6 per cent solution, while it is 2 weeks longer for both the 1 per cent and the 0.3 per cent solutions. In the later stages of the experiment the differences due to dosage become

Table I

Frequency of application of 0.6% methylcholanthrene solution in benzene	Amount of methylcholanthrene applied before appearance of cancer		
	In first mouse, mgm.	In 50% of mice, mgm.	In 100% of mice, mgm.
Routine technic:			
3 times weekly	1.8	4.2	4.2
Protracted technic:			
C once in 2 weeks	0.6	1.2	1.7
D once in 3 weeks	0.5	0.8	1.1
E once in 4 weeks	0.3	0.6	0.9

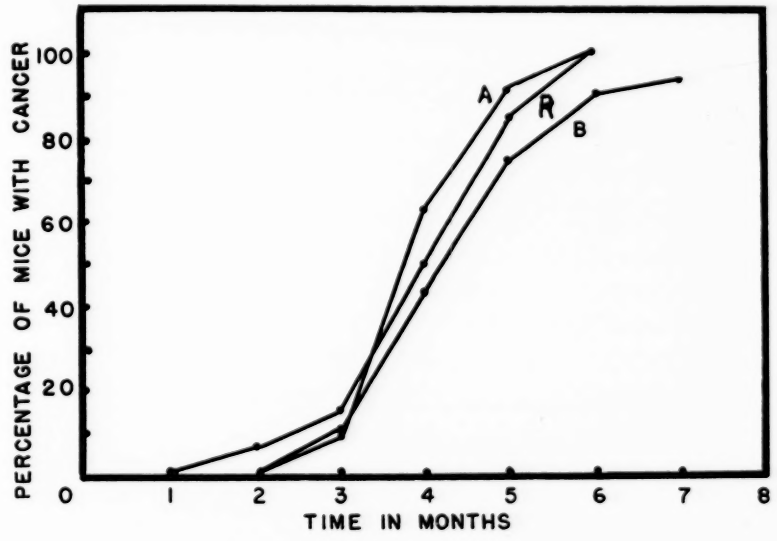


Fig. 3.—Incidence in mice of skin cancer, relative to time, following continuous exposure to varying concentrations of methylcholanthrene for 14 weeks, 1 per cent (A), 0.3 per cent (B), and 0.6 per cent (R).

more definite and the development of cancer in the last 25 per cent of the animals is distinctly delayed in the group receiving the 0.3 per cent solution.

If the results were to be recorded with the dose of carcinogen as abscissa instead of the time of appearance of a malignant tumor, the first tumors would have to be entered as appearing after the following amounts of carcinogen have been applied:

For the 1 per cent solution after 30 applications = 4.5 mgm.
For the 0.6 per cent solution after 24 applications = 2.4 mgm.
For the 0.3 per cent solution after 30 applications = 1.5 mgm.

To record these graphs in Fig. 2, for purposes of comparison with the method of discontinuous exposure, it would be found that the graphs for the 1 per cent and the 0.6 per cent solution would begin outside that figure while the graph for the 0.3 per cent solution would begin in its lower right hand corner. In other words the amounts of carcinogen applied in the continuous method of exposure before the appearance of the first skin carcinomas are as large as, or larger than, those required to produce skin carcinomas in 100 per cent of the animals if applied by the discontinuous method of exposure.

Limited Carcinogenesis

We may now consider the question: What is the carcinogenic effect when small doses of the carcinogen, similar to the smallest dose of methylcholanthrene sufficient to induce skin cancer in 100 per cent of the animals by the discontinuous method of exposure over a long time as in experiment E, are applied to the skin continuously over a short time? The answer to that question is given in three experimental series labelled respectively F, G, and H, in which a 0.6 per cent solution of methylcholanthrene in benzene was applied for short periods of time under slightly varying conditions. The experimental details and the results are given in Table II and Fig. 4. In experiments F and G the carcinogen was applied 3 times a week, which ensured a continuous exposure. In experiment F this exposure extended to 4 weeks, in experiment G to 2 weeks. In experiment H, 3 applications were made at intervals of 3 weeks so that the exposure was discontinuous. The effects of a single application with 3 brush strokes (8) are also included in Table II and Fig. 4 (experiment S).

It will be seen that compared with the total dose given in the routine technic (4.2 mgm.) the total doses of the carcinogen administered in experiments F, G, and H were small and the time of exposure was limited to periods varying from 2 to 6 weeks instead of 14 weeks as in the routine technic. Nevertheless more than 40 per cent of the animals developed malignant tumors, mostly carcinomas but also a few sarcomas. If analyzed according to time the earliest tumors appeared at about the same time as in the routine method with much larger doses, but the development of the subsequent tumors is considerably

Table II: Carcinogenesis by Small Doses of Methylcholanthrene

Mode of application	F 3 times weekly for 4 weeks	G 3 times weekly for 2 weeks	H 3 paintings, once every 3 weeks First painting with 3 brush strokes	S Single application with 3 brush strokes
Total dose of methylcholanthrene	1.2 mgm.	0.6 mgm.	0.5 mgm.	0.3 mgm.
Effective number of mice	20	20	34	14
Mice with carcinomas	6, *30%*	6, *30%*	12, *35%*	5, *35%*
Mice with sarcomas	3, *15%*	3, *15%*	2, *6%*	1, *7%*
Total number of mice with malignant tumors	9, *45%*	9, *45%*	14, *41%*	6, *42%*
Period of induction:				
First tumor	3 months	3 months	4 months	3 months
Last tumor	7 months	10 months	9 months	10 months

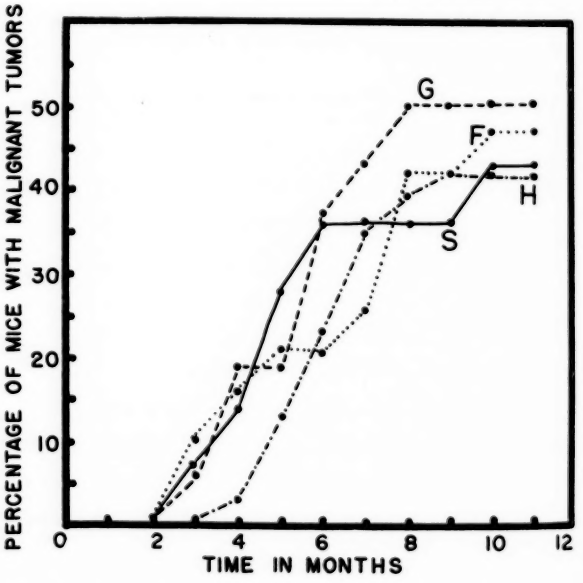


Fig. 4.—Limited carcinogenesis by small doses. Incidence in mice of skin cancer, relative to time, after treatment with 0.6 per cent methylcholanthrene 3 times weekly for 4 weeks (F) and for 2 weeks (G), once every 3 weeks for 3 times (H), and a single painting (S).

delayed. The carcinogenic response is, in fact, similar to that obtained after a single exposure to a dose of 0.3 mgm. (graph S in Fig. 4). In view of the small number of effective mice used in experiment S, the percentage figure is given provisionally. While this paper was being prepared for publication another experiment carried out in this laboratory by Dr. W. L. Simpson and the senior author has been completed. Its object was to establish with certainty the discontinuity of exposure of the skin to the carcinogen, when it is applied at intervals of 3 weeks. At this interval 5 applications were made by a single brush stroke, but before each application 1 mouse of the group was killed and frozen sections of the skin were examined by ultraviolet microscopy. No fluorescence was visible in ultraviolet light in any of the animals, so that the absence of methylcholanthrene was established before each new application was made. The total dose administered in 5 applications was 0.5 mgm., the same as in experiment H. Of 41 effective mice 17 had developed skin cancer after 10 months, *i.e.* 41 per cent, a percentage identical with that obtained in experiment H. This experiment together with experiments F, G, and H shows that under our experimental conditions the carcinogenic response of the skin of Swiss mice to a small total dose of methylcholanthrene is remarkably constant, whether the carcinogen is applied continuously or discontinuously.

Multiplicity of the Carcinogenic Response and Its Relation to Dosage

This apparent failure in the carcinogenic process to respond to considerable changes in dosage, when the process is measured in time, finds an explanation in differences in the type of carcinogenesis induced by different dosages. These differences will now be considered.

Variations in the technic of applying the carcinogen affect not only the rapidity with which cancer develops and the percentage of animals in which it appears, but also some clinical and pathological features of the disease. Clinically there are differences in the frequency with which multiple skin carcinomas develop; pathologically there are differences in the condition of that area of the painted skin in which the carcinomas have appeared, and in the histological type of the epithelial neoplasms. For the elucidation of the problem under consideration it is the presence or absence of multiple skin carcinomas that is of chief importance and that will now be considered. Table III contains in the vertical columns the results of nine different experiments, which all differ from each other in some feature of the technic of application of the carcinogen. The table is based on data obtained from 291 effective mice, of which 228 had one or more carcinomas. Each experiment has at the top of the vertical column a capital letter from A to I as reference. Details of experiments B and I have been published (14, 6) and will not be referred to again here. Experiments C through H have been described in this communication. The table has been arranged in such a manner that as one passes from experiment A to experiment H the number of applications, *i.e.* the total dose of the carcinogen, diminishes.

In following the arrangement of the table from the top downwards the first two groups to be contrasted are:

1. Painting of a large and of a small skin area.—The small skin area was a circle with a diameter of about 6 mm. In this small-area experiment the amount of methylcholanthrene delivered to the skin at each application was 0.02 mgm. as determined by the device described in a previous paper (5). The total dose after 42 applications made 3 times a week thus amounts to 0.8 mgm.

The small skin area is represented by experiment I. The remaining 8 experiments, in which a large skin area was exposed to the carcinogen, fall again into two groups.

2. 100 per cent carcinogenesis and limited carcinogenesis.—The term "100 per cent carcinogenesis" means that the carcinogen is applied sufficiently often to ensure carcinogenesis in all the mice alive at the end of the experiment. The term "limited carcinogenesis" means that applications are restricted in number so that only a fraction of the animals develop cancer. Each one of these two groups is divided into two subgroups, described as:

3. Continuous and discontinuous exposure.—Continuous exposure of the skin means that the carcinogen is applied at such short intervals of time, namely 3 times weekly, that the skin is continuously exposed to the action of the carcinogen. Discontinuous exposure indicates a technic in which the application of the carcinogen is made at intervals of time sufficiently long to ensure that the carcinogen has disappeared from the skin before a subsequent application is made. In these experiments with discontinuous exposure these intervals of time are different in the various experiments.

In Table III these technical details are given in the first horizontal entry. The second horizontal entry indicates the total number of applications given in each experiment, the third the total dose of carcinogen administered as calculated from the number of applications, and the fourth the concentration of methylcholanthrene in benzene. This concentration was the same, 0.6 per cent for all experiments, except for the first two, A with a 1 per cent solution, and B with a 0.3 per cent solution. These two experiments, A and

B, are included to show the effect of differences in the dose of carcinogen administered at each individual application. The 0.3 per cent solution has been selected from our experiments for inclusion in the table because this is the concentration used by most workers.

This completes the explanation of the details of technic for each experiment. Underneath the headings the results are arranged according to the number of mice found to have 1, 2, 3, or 4 carcinomas respectively. The figures are given both for the numbers actually observed and, next to it, in percentage of the if 100 cancerous mice had been obtained in each experiment. These figures were entered in the last horizontal entry opposite "No. of cancers in 100 cancerous mice." The figure 100 in this line would indicate that all the cancerous mice had developed only one carcinoma each. The figures above 100 give the average incidence of multiple skin carcinomas. Thus in experiment A the figure 209 signifies that the animals in this experiment had an average of slightly more than 2 carcinomas per mouse. The figures 167 in experiment D and 147 for experiment E signify

TABLE III

	Large area								Small area
	100% carcinogenesis					Limited carcinogenesis			
	Continuous exposure		Discontinuous exposure			Continuous exposure		Discontinuous exposure	Continuous exposure
	A	B	C	D	E	F	G	H	I
Frequency of application	3 times weekly	3 times weekly	1 time in 2 weeks	1 time in 3 weeks	1 time in 4 weeks	3 times weekly for 4 weeks	3 times weekly for 2 weeks	1 time in 3 weeks	3 times weekly
No. of applications	42	42	17	12	9	12	6	3	42
Total dose	6 mgm.	2 mgm.	1.7 mgm.	1.2 mgm.	0.9 mgm.	1.2 mgm.	0.6 mgm.	0.5 mgm.	0.8 mgm.
Concentration of methylcholanthrene	1 %	0.3 %	0.6 %	0.6 %	0.6 %	0.6 %	0.6 %	0.6 %	0.6 %
No. of carcinomas in individual mice	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %
1	6, *33*	20, *53*	16, *42*	24, *55*	33, *70*	5, *83*	5, *83*	10, *83*	17, *94*
2	6, *33*	12, *31*	17, *45*	12, *27*	9, *19*	1, *17*	1, *17*	2, *17*	1, *6*
3	4, *22*	3, *8*	4, *10*	6, *14*	5, *11*	—	—	—	—
4	2, *11*	3, *8*	1, *3*	2, *4*	—	—	—	—	—
Total No. of:									
(a) Cancerous mice	18	38	38	44	47	6	6	12	18
(b) Cancerous tumors	38	65	66	74	66	7	7	14	19
No. of cancers in 100 cancerous mice	209	171	174	167	141	117	117	117	106

total cancerous mice. Sarcomas were not included. When a carcinoma was accompanied by a sarcoma it was entered as a single carcinoma. Only fully developed carcinomas were entered as such; precancerous papillomas and other precancerous changes were not included.

From these records figures were obtained and entered in the last 3 horizontal entries opposite (a) the "total number of cancerous mice" and (b) the "total number of cancerous tumors" in the cancerous mice. Since the number of cancerous mice varied in the different experiments it was necessary for purposes of comparison to calculate from (a) and (b) the number of cancerous tumors that would have been present that in experiment D the animals had an average of 1.67 carcinomas, in experiment E an average of 1.47 carcinomas per mouse. The actual distribution in individual animals of multiple skin carcinomas in the various experiments is shown under the heading of "No. of carcinomas in individual mice."

In Table III experiments A to H, which refer to large skin areas, are arranged as already stated in such a manner that as one goes from left to right the total dose of carcinogen applied and the number of applications diminish. The table shows that as this diminution proceeds from experiment A to experiment H it is accompanied by a diminution in the number of multiple skin carcinomas. The average incidence

of multiple skin carcinomas as shown by the figures in the last line, "No. of cancers in 100 cancerous mice," falls steadily from 209 in A to 141 in E. In experiments A to E cancer was induced in all the surviving animals, experiment E representing conditions of the minimal dose and the minimal number of applications with which the 100 per cent result has so far been obtained by us. The average incidence of multiple skin carcinomas falls further in experiments F, G, and H, in which a still smaller dose and a still smaller number of applications induced carcinomas but only in a fraction of the experimental animals.

These differences are even more pronounced when the actual distribution of skin carcinomas in the various experiments is considered. The number of mice with a single carcinoma steadily rises and the number with 4 carcinomas steadily falls from A to D, until animals with 4 carcinomas fail to appear in experiments E to H. In experiments F to H, belonging to the group of experiments in which limited carcinogenesis was practiced by means of a few applications only, there are no animals with 3 carcinomas and very few with two. The last vertical column (experiment I) represents the result of a continuous exposure of a small skin area (a circle of about 6 mm. diameter) to methylcholanthrene over the same period (14 weeks) as used in experiments A and B. Only about 30 per cent of the effective mice developed malignant tumors. The incidence of multiple carcinomas is also very low, although 42 applications were given. This is mainly due to the fact that in such a small skin area there is no room for the development of more than one carcinoma. With the concentration of the methylcholanthrene solution in benzene (0.6 per cent) used, the amount delivered to such a small area at each application was much smaller (0.02 mgm.) than that delivered to a large area (0.1 mgm.). This difference in the amount of carcinogen delivered to a large and to a small skin area is roughly proportional to the differences in size of the two areas, so that the amount of methylcholanthrene per area of skin was about the same. As pointed out previously the size of the skin area that is exposed to a carcinogen is a factor in determining the carcinogenic response.

It is evident that the biological assay of chemical carcinogens by the number of cancerous mice, regardless of the number of malignant tumors they carry, does not tell the whole story. If, instead, the number of carcinomas arising in the treated mice is recorded, as in Fig. 5, a very different picture is obtained. Then the reduction, in the total dosage from 6 mgm. in experiment A to 2 mgm. in experiment B, brought about by a diminution in the concentration of the methylcholanthrene solution, and the further reduction to 0.9 mgm. in experiment E, resulting from a diminution in the number of applications, are reflected in diminutions in the carcinogenic response corresponding more closely to the changes in dosage.

The Successive Development of Multiple Carcinomas

We wish to point out here that even this method of evaluating the carcinogenic response is not yet adequate. We have restricted ourselves to the carcinomas that develop simultaneously in individual

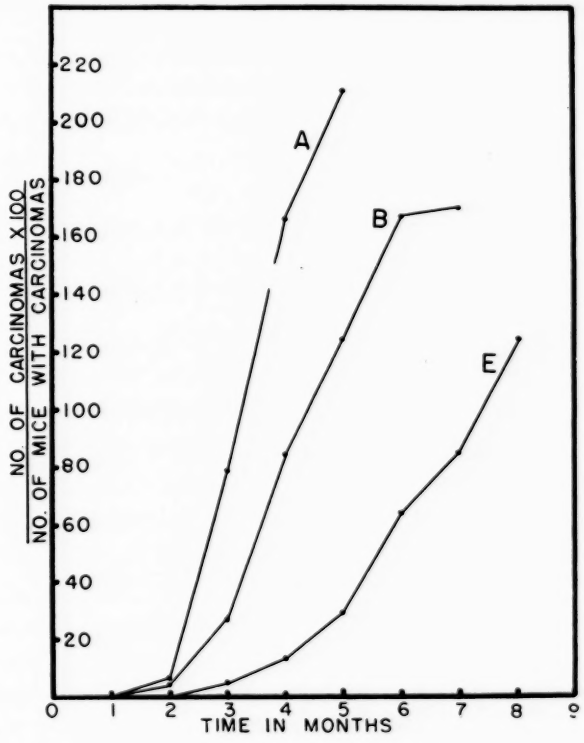


Fig. 5.—Incidence of total numbers of epidermal carcinomas, calculated on the basis of 100 cancerous mice, following painting with 1 per cent (A) and 0.3 per cent (B) methylcholanthrene thrice weekly and with 0.6 per cent methylcholanthrene at monthly intervals (E).

mice. When relatively large doses of methylcholanthrene have been administered so that skin cancer develops in 100 per cent of the surviving mice there are frequently also papillomas, often very numerous, some of which on microscopic examination prove to be precancerous and there are in addition, in parts of the treated skin, areas of nonpapillomatous precancerous hyperplasia. Earlier work in which a large skin area was painted with tar has shown that by removing the carcinomas by operation soon after they had developed and allowing the animals to survive, a successive development of further carcinomas sets in (4). In other words, we have not taken into account the successive development of multiple skin

carcinomas when large doses of the carcinogen have been applied to an extensive skin area. The inclusion of these successive multiple carcinomas would therefore greatly emphasize the differences in the carcinogenic response discussed above, as will be shown presently.

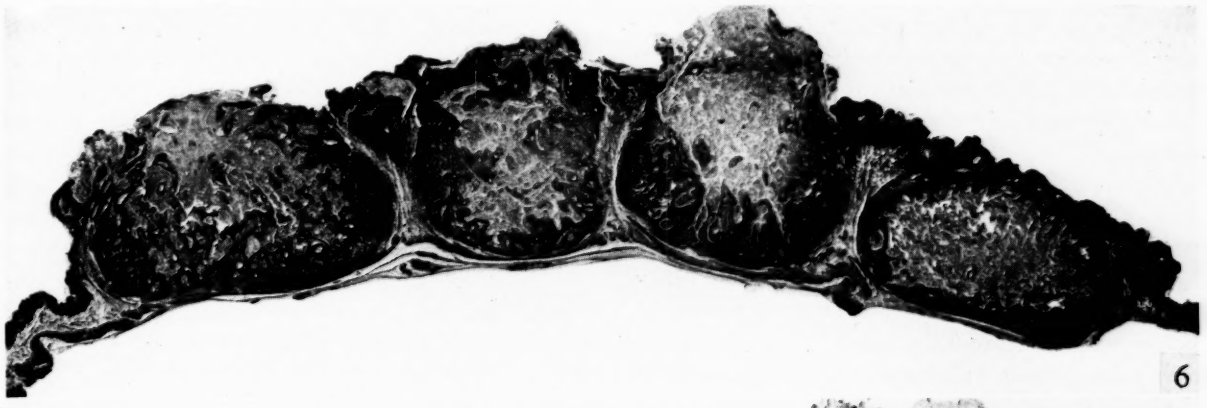
Further information on the conditions determining the development of multiple skin carcinomas is obtained from a consideration of the experiments described earlier in this paper in which the carcinogen has been applied to the skin for only a short period (experiments F, G, and H, Table II). Under these conditions the development of multiple precancerous skin conditions in addition to a fully developed skin cancer is much less frequent. Thus in experiment G a 0.6 per cent benzene solution of methylcholanthrene was applied to the skin of 20 mice 3 times weekly for 2 weeks. In the course of time 6 mice developed carcinomas—these are the mice with a "susceptible" skin—2 developed sarcomas, while the remaining 12 mice remained negative. But in the 6 susceptible mice the tumors did not develop with equal rapidity. The shortest period of induction was 3 months, the longest 10 months. Similarly after a single application of the carcinogen (experiment S, Table II) the last skin cancer appeared 10 months after the application. Even in susceptible mice, therefore, the period of induction can be very prolonged. The existence of such prolonged periods of induction after the application of relatively large doses of a potent carcinogen has not been generally recognized hitherto, because in the routine method of continuous exposure to massive doses of the carcinogen all the animals have developed skin cancers after an interval of 6 months from the first application and only 3 months after the last application. When a presumably malignant tumor has developed the animals are killed for microscopic examination and confirmation of the diagnosis. The following considerations afford an explanation of this phenomenon.

In 5 of the 6 susceptible mice of experiment G only one carcinoma developed, while the skin outside the carcinomatous area was devoid of hyperplastic changes. It was, in fact, almost normal. This means, then, that not the whole skin but only a small area of the skin of these mice has been "susceptible" to the carcinogenic effect of the methylcholanthrene.

The differences in the carcinogenic response of the skin to different technics of the application of the carcinogen are illustrated in Figs. 6 to 9. Fig. 6 and Fig. 7 represent sections of the skin of mice that had been painted with a 1 per cent solution of methylcholanthrene in benzene 3 times a week for 14 weeks (experiment A). Fig. 6 is from a mouse killed 1 week after the last application. The whole painted skin area was covered with papillomas and cutaneous horns, piled up against each other. Four separate areas were removed and examined microscopically. As shown in Fig. 6, in one of these areas there were 4 papillomas all in a row, of which the 2 outer ones had become malignant. Two other fully developed carcinomas were present in the other areas, in addition to several precancerous papillomas. Fig. 7 is from another animal of the same experiment killed 1 week after the last application. The skin presented 2 large tumors and a number of smaller papillomatous growths and cutaneous horns. In 4 separate areas removed for microscopic examination 2 large and 2 small carcinomas were found and in addition a number of areas of precancerous epidermal hyperplasia. At the right end of Fig. 7 is the edge of one of the large carcinomas; the skin in the central part is hyperplastic, hyperkeratinized, and covered with small papillomas, while at the left end of the section there is part of a small carcinoma. Altogether 4 carcinomas were present in the skin of this animal.

Fig. 8 represents the skin of a mouse that had been subjected to the protracted method of applying the carcinogen; *i.e.,* infrequent application at long intervals of time, repeated until a carcinoma develops (experiment C). In Fig. 8 the skin had been exposed to a 0.6 per cent solution of methylcholanthrene in benzene once every 2 weeks for 26 weeks, so that 13 paintings had been given. The skin had shown no obvious change apart from epilation for 18 weeks. Then a shallow ulceration developed, which grew in circumference and slightly in depth, and a few small warty growths appeared. The mouse was killed 28 weeks after the first application. The section is axial and therefore goes through the length of the skin area exposed to the carcinogen. There is an anaplastic carcinoma at the left end of the section, while the remainder of the epidermis shows a massive epidermal hyperplasia with several precancerous centers.

In Fig. 9 the opposite condition is represented by the skin of a mouse that had received one single application of the carcinogen. A tumor was first seen 13 weeks afterwards and diagnosed as malignant a week later. The mouse was killed 2 weeks later, 16 weeks after the single application. The period of induction is, therefore, almost the same as for the skin conditions shown in Figs. 6 and 7. Three areas of the skin were examined in sections, two transverse and one axial. Fig. 9 is taken from the axial section so that it runs through the whole length of the area that had been exposed to the carcinogen. The growth was a very anaplastic carcinoma that had developed on the back of the neck; *i.e.,* the upper limit of the painted area. Apart from this the skin was normal to the naked eye and microscopically. Hair follicles and



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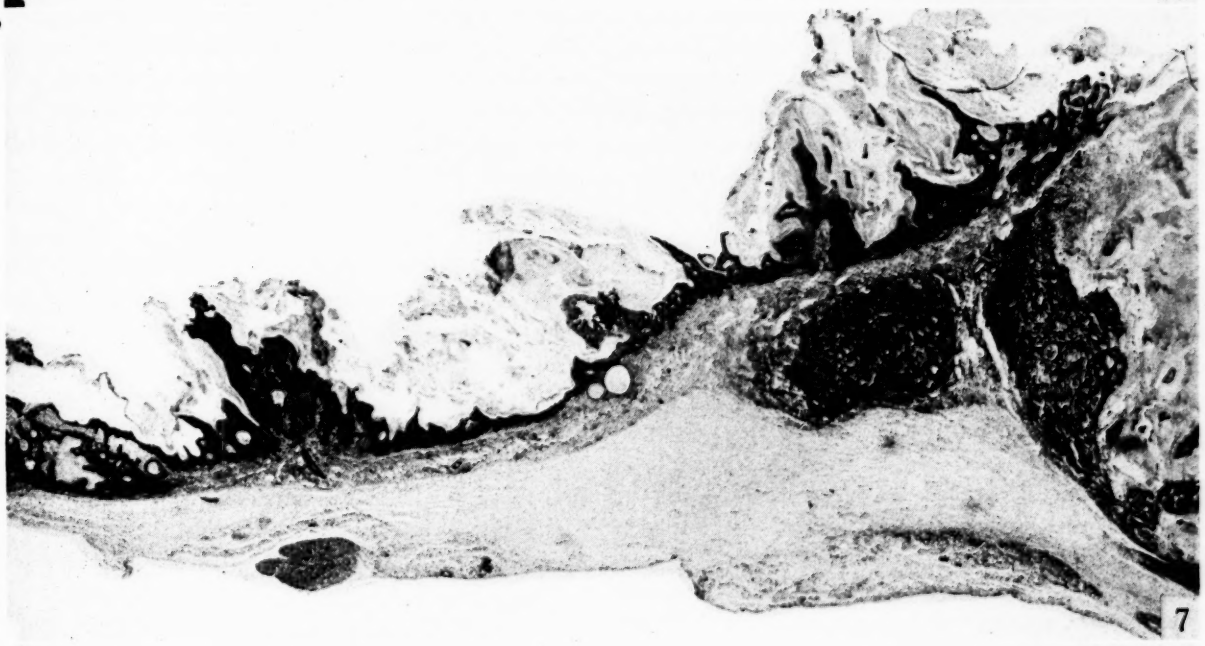

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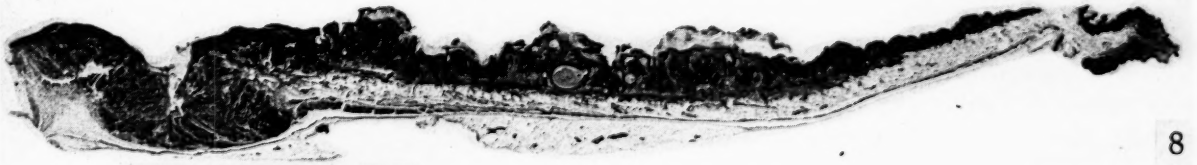

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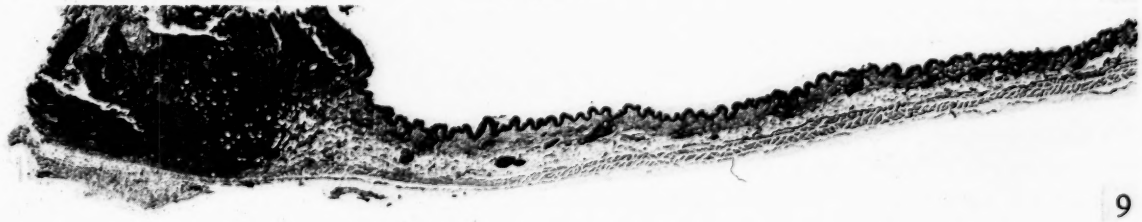

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FIGS. 6 to 9 illustrate differences in the carcinogenic response of the skin of mice owing to differences in the dosage of the carcinogen.

FIGS. 6 and 7.—Skin of 2 mice from experiment A. Continuous exposure to methylcholanthrene produced by applications made 3 times weekly for 14 weeks. Total dose, 4.2 mgm. Mag. × 6 and × 10.

FIG. 8.—Skin of mouse from experiment C. Discontinuous exposure produced by 13 applications made once every 2 weeks within 26 weeks. Total dose, 1.3 mgm. Axial section. Mag. × 8.

FIG. 9.—Skin of mouse from experiment S. Single application in 3 brush strokes. Total dose, 0.3 mgm. Axial section. Mag. × 12.

sebaceous glands were present and there were no hyperplastic or hyperkeratotic conditions. There is an abrupt transition from the normal epidermis to the carcinoma. Fig. 9 is fairly typical of the appearance of the skin when it has been exposed to only a few applications of the carcinogen, resulting in a limited carcinogenesis as in experiments F, G, and H. Figs. 6, 7, and 8, on the other hand, have been selected to illustrate extreme results that have been seen after frequently repeated observations leading to a 100 per cent carcinogenesis. But it must be borne in mind that in some very resistant mice, even after exposure of the skin to frequently repeated applications of the carcinogen, the epidermal hyperplasia on the basis of which a carcinoma develops is sometimes sharply circumscribed and the remainder of the skin shows few or no hyperplastic changes. A figure illustrating such a condition has been published by us in a previous paper (7, Fig. 2). The results may be summarized as follows: The conditions illustrated in Figs. 6, 7, and 8 were seen frequently after large total doses of the carcinogen had been administered over a long period as in experiments A to E. They have never been observed after the application of small doses over a short period as in experiments F to H. In these latter experiments the skin frequently showed the condition illustrated in Fig. 9; it has been seen occasionally but infrequently in experiments H to E.

The differences in the carcinogenic response of the skin are of importance in interpreting the significance of the experimental production of multiple skin cancers. *A priori* one might postulate that in a susceptible mouse the whole area of skin exposed to the carcinogen is fairly uniformly susceptible to the chemical carcinogen. In that case multiple cancers should develop in the susceptible mice when limited carcinogenesis is practiced by means of a few applications. The facts are otherwise. We must conclude, therefore, that in the group of mice selected as susceptible by a small dose of the chemical carcinogen administered in a few applications, only a small area of the skin is susceptible. When as the result of numerous applications involving more massive doses multiple cancers appear at about the same time, they are actually the result of several independent processes of carcinogenesis induced successively in separate small areas of individual animals. These independent, successive processes of carcinogenesis may lead to the development of multiple carcinomas. But since, after periods of induction varying greatly in length, the experimental animals are killed whenever one mouse has developed at least one tumor of unquestionable malignancy, only the multiple carcinomas with the shortest periods of induction came under observation. The routine method of carcinogenesis is therefore one which, by favoring the development of multiple skin cancers, selects carcinomas with the shortest periods of induction. These facts account, partly at any rate, for the shortening of the period of induction when skin cancer is induced in mice by the routine method of continuous exposure as compared with the method of discontinuous exposure of the carcinogen. There may be, in addition, an acceleration in the development of skin carcinomas. But such an acceleration is demonstrable only in the later stages of an experiment, that is to say it is restricted mainly to resistant individuals, while the development of the first skin carcinomas, which picks out the susceptible individuals, occurs at about the same time after a single application and after frequently repeated applications. Thus in one experiment with a 0.6 per cent solution of methylcholanthrene the shortest period of induction for the first carcinoma is 2 months with frequently repeated applications, and 3 months in our published experiment on carcinogenesis after a single application (8). In an experiment with a single application carried out recently with Dr. W. Simpson the earliest carcinomas appeared after 2 and $2\frac{1}{2}$ months respectively.

DISCUSSION OF RESULTS

It has been established that the continuous exposure of a skin area to a carcinogen, which is the routine method of experimental carcinogenesis, is not essential for the induction of skin cancer in mice. With a potent carcinogen applied to a large skin area it is possible to induce skin cancer in 100 per cent of the experimental animals by a method involving a discontinuous exposure. In this technic the carcinogen is applied at intervals of time longer than the period during which the carcinogen persists in the skin after each application. The intervals of application tested successfully so far are 2 weeks, 3 weeks, and 4 weeks. This method of discontinuous exposure requires the application of a much smaller total dosage of the carcinogen to obtain a 100 per cent effect than the method of continuous exposure. Furthermore the total dosage becomes increasingly smaller the longer the time intervals of applications.

If the total dosage of the carcinogen is further reduced by diminishing the number of applications and restricting them to a short period of time cancer can also be induced in the skin, but then only in a fraction of the experimental animals. Even a single application of the carcinogen is sufficient to induce cancer in a fraction of the experimental animals.

The implications of these facts on our conception of the mode of action of chemical carcinogens are that the development of skin cancer cannot readily be attributed to a direct growth-stimulating action of the carcinogen on the epithelial cells. As pointed out in

a previous paper (7) it can be accounted for hypothetically by the action of a new substance formed in the skin under the influence of the carcinogen. It is interesting to note that recently Mottram and Weigert (10) have been able to demonstrate in the skin of mice treated with benzpyrene the presence of a water-soluble fluorescent substance, probably a benzpyrene derivative. Their observations agree with those of Simpson and Cramer (13) in establishing the fact that the carcinogen itself disappears from the skin after an interval of 6 to 10 days following the application. But the new water-soluble fluorescent substance observed after the application of benzpyrene persists in the skin for weeks.

A number of methods are now available for inducing skin cancer experimentally by means of a chemical carcinogen. They differ in the dosage of the carcinogen, the number of applications, the spacing of the applications, and the period of time during which the skin area is exposed to the action of the carcinogen.

It is now possible to produce at will the development of a single carcinoma or of multiple carcinomas by choosing a suitable method of application. The experimental conditions mentioned below apply to the use of a potent carcinogen such as methylcholanthrene in concentrations varying from 0.3 to 1 per cent and applied to a relatively large area of the skin of the back. The dosage can be obtained from the number of applications. A single brush stroke of a definite length delivers to the skin a dose of 0.1 mgm. when a 0.6 per cent concentration is used and correspondingly different amounts for the higher and lower concentrations. Single carcinomas are induced when the number of applications is low and when, therefore, the total dose of carcinogen applied is small, from 0.6 mgm. to 1.0 mgm. If a few applications are given at prolonged intervals over a long period of time skin cancers are induced in 100 per cent of the treated animals and most of them are single. If the same number of applications are made at short intervals but restricted to a short period of time only a fraction of the mice develop skin cancers and most of them are single. Multiple cancers are induced when the applications are frequent and when, therefore, the total dose is high.

The fact that with small doses only a fraction of the animals respond to the carcinogen with the development of skin cancer indicates that in one and the same strain different individuals show greatly varying degrees of susceptibility to the carcinogenic effect. The further fact that in these susceptible individuals only a small area of the skin responds to a single dose or a few doses of the carcinogen with the development of a single carcinoma shows that even in these susceptible individuals only a small area of the skin is susceptible. The main portion of the treated skin is resistant and requires larger doses, *i.e.* more numerous applications, for the induction of cancer. The development of multiple skin cancers is, therefore, initiated at different times and represents several independent processes of carcinogenesis. The correctness of this conclusion is confirmed by the fact that when the carcinogenic response is measured by the number of cancerous tumors and not by the number of cancerous animals an increase in dosage is reflected in an increase in the carcinogenic response.

The conditions that favor the development of multiple skin cancers induce also various forms of precancerous skin conditions such as cutaneous horns, papillomas, and massive, nontumorous, epithelial hyperplasias. Such conditions are only infrequently found when single carcinomas are induced by the use of small doses.

When a 100 per cent carcinogenic effect is produced by a small total dosage the period of induction is not much delayed for the early tumors as compared with the carcinogenic effect induced by the use of large dosages, but it is greatly prolonged for the late tumors. When the routine method of carcinogenesis is used, in which frequent applications are made, involving a large dosage, the development of cancer is, therefore, apparently accelerated, but the acceleration affects mainly the group of resistant individuals.

These facts demand a reconsideration of the methods now in use for measuring carcinogenic potency. As stated at the outset of this paper the generally accepted method of producing a carcinogenic response in the mouse skin is to administer to the surface of the skin by frequent applications, repeated at short intervals over a period of several months, a large total dose of the carcinogen, so as to obtain the largest possible number of cancerous animals within the shortest possible time. The carcinogenic response is then measured by the ratio of the number of cancerous animals to the average period of induction, the dosage remaining the same for different carcinogens. The two determining factors are the percentage of cancerous animals and the time of induction. If the carcinogens are potent the percentage of animals will be 100 and the time of induction becomes directly the measure of the carcinogenic potency: the shorter the time the greater the carcinogenic potency. As a result there has been a tendency in recent years to work under conditions that are likely to shorten the period of induction. This has led to the method of measuring carcinogenic potency by determining quantitatively the power of a carcinogen to induce sarcomas after subcutaneous injection, and for this method Fieser (9) has suggested the determination of the "maximum effective dose," *i.e.* the dose producing the greatest

incidence in the shortest possible time, as a measure of carcinogenic potency. There are, however, objections to identifying the "sarcogenic" potency of a chemical substance with its "carcinogenic" potency. The same carcinogen does not necessarily react in the same way on different tissues, and for the same carcinogen a high susceptibility of one tissue is not necessarily associated with a high susceptibility in other tissues. There are other objections that need not be discussed here.

The observations recorded in this paper make it questionable whether it is desirable to shorten the period of induction by increasing the dosage. Such a method has the obvious advantage that it shortens the experiment and thus is useful in determining qualitatively whether and to what extent a substance is carcinogenic. But as a quantitative method it fails. The carcinogenic response does not increase proportionally to the increase in dosage, and no account is taken of the multiplicity of the carcinogenic response in individual animals. This latter objection can be overcome, partly at any rate, by counting the number of malignant tumors developing in individual animals, instead of counting the number of cancerous animals. It could be improved even further if the animal were kept alive by operative removal of the cancerous growth that develops first. This would allow any precancerous conditions that exist to progress to the development of successive multiple growths. But such a procedure would be too cumbersome to make this a useful method for the quantitative evaluation of the process of carcinogenesis. As a rule the mode of action of a biologically active substance is not studied by applying maximal effective doses, nor is their activity measured by the time in which they produce their effect. A method that aims at ironing out differences between individual animals by massive doses, and differences between different areas of a given tissue in one and the same animal may appear to simplify the problem. But such a simplification is deceptive, and obscures rather than reveals the complexity of factors involved in the process of carcinogenesis.

Another method—and in our opinion a better one—of studying the mode of action of active carcinogens and determining quantitatively their potency is based on the use of minimal effective doses. We would define a minimal effective dose provisionally as a dose that, when applied to a large skin area, induces mainly single carcinomas. There are various ways in which minimal doses can be used to measure quantitatively carcinogenic potency. From the results of our experiments the two following methods appear to be suitable. In one the carcinogen is applied at long intervals (once a month) until all the animals have developed skin cancer. The carcinogenic potency is then measured by the total dose of the carcinogen as represented by the number of applications. This method is suitable for potent carcinogens. In the other a limited number of applications, *e.g.* 6, is made at intervals of 3 weeks. The carcinogenic potency is then measured by the number of skin cancers that develop, the dosage being kept the same. The second method has the advantage that the period during which the carcinogen is applied is limited. It should be suitable for the experimental study of conditions that enhance or inhibit the effect of the carcinogen either during the period of its application or after that period. In both these methods the time factor is of secondary importance. The experimental investigation of factors that enhance or impair the carcinogenic action of chemical carcinogens requires the use of minimal doses. The existence of such factors is likely to be obscured by the use of massive doses. In so far as such factors may be due to physiological or pathological changes in the skin they would reveal the conditions on which susceptibility or resistance of the skin to cancer depends and which, therefore, determine the onset of the disease in man. From this point of view it should be our aim to make experimental skin cancer resemble the disease in man as closely as possible.

In man skin cancer generally develops as a single growth and in only a fraction of the persons exposed to a carcinogenic agent such as the actinic rays of the sun. When multiple skin cancers develop they may appear either simultaneously or successively. In this hospital, of 1,790 cases of cancer of the skin, excluding the lip, vulva, penis, and anus, observed from 1936 to 1941, 106 patients, or 6 per cent, had multiple cancers (2). A more frequent occurrence of multiple skin cancers has been noted in Texas, where in a group of 1,400 persons with skin cancer 226 patients or 16 per cent had multiple skin cancer (12). These 226 patients had a total of 704 individual skin cancers, almost all on sites exposed to light. Eighty-five per cent of the multiple cancers were in persons over 50 years of age. One of the patients had as many as 23 microscopically verified epitheliomas, another 26. From our experimental observations the development of multiple skin cancer in man can be attributed to the frequent exposure of a large skin area to a potent carcinogenic agent, such as sunlight, over a long period of time. Where the sunlight is less potent and exposure to it less prolonged, as it is in the Northern States, multiple skin cancers are less frequent.

SUMMARY AND CONCLUSIONS

Further evidence is adduced to show that a continuous exposure of the mouse skin to methylcholanthrene, which involves the application of relatively large total doses in order to induce skin cancer, is not

essential. Skin cancer can be induced by much smaller doses if the applications are made at long intervals such as one month. This implies a discontinuous exposure of the skin to the carcinogen.

These facts necessitate revising current conceptions on the mode of action of chemical carcinogens. In discussing these implications the following conclusions have been drawn. Increasing the dosage applied to a large skin area increases the number of multiple skin cancers. Minimal dosages induce single skin cancers even in the more susceptible individuals of a strain. A multiplicity of skin cancer is therefore not in itself an indication of an increased susceptibility of the skin to a carcinogen.

Increasing the dosage shortens the time at which cancer develops in resistant animals; it does not materially shorten the time of induction in the most susceptible animals. The quantitative evaluation of the carcinogenic potency of chemical substances should be based on the use of minimal doses of the carcinogen. It can be determined by the percentage of cancerous tumors and the dose, rather than by the percentage of cancerous animals and the time of induction.

The fact that the exposure of a large area of skin to minimal effective doses induces cancer in only one small area of the exposed skin, even in the susceptible individuals, indicates that a high susceptibility to skin cancer does not necessarily extend over the whole skin but may be restricted to one small area.

The etiology of multiple skin cancer in man is discussed in the light of our findings.

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Experimental Brain Tumors

III. Tumors Produced with Dibenzanthracene*

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The unexpected success with benzpyrene in producing intracranial neoplasms in C3H mice (6), after many unsuccessful attempts by other workers, was a clear indication to try still another chemical carcinogen for its effect on brain tumor production. The carcinogen chosen was 1,2,5,6-dibenzanthracene.

In 1938, Weil (3) reported the result of injecting a lard solution of dibenzanthracene into the brain of a white rat. He stated that 7½ months after the injection he found a squamous epithelial carcinoma and surrounding the tumor a definite glial proliferation. In the summary of his findings Weil wrote, "One case was noted in which injection of the dibenzanthracene-lard mixture produced a malignant carcinoma besides granuloma." No mention was made of a glioma produced with dibenzanthracene yet, in 1941, in discussing a paper by Sweet and Bailey (2) on the experimental production of intracranial tumors with methylcholanthrene, he referred to this animal as having both an epidermoid carcinoma and a glioma. The illustration (Fig. 1-A) in the original report of 1938 shows perhaps some glial proliferation but is decidedly not convincing as a glioma.

More recently Weil and Blumklotz (4) redescribed this case and stated that "In the midbrain, tissue posterior to this epithelial tumor shows neoplastic transformation into a glioma." There is certainly evidence of glial proliferation in the illustrations (Figs. 5-C and 7) of this part of the brain but it is, nevertheless, questionable whether this constitutes gliogenous neoplasia.

The most extensive animal experiments with intracranial implantation of dibenzanthracene were performed by Peers (1), who employed cholesterol pellets containing 5 per cent of this carcinogen. Of 53 stock albino mice that survived 6 months or more, not a single animal developed a definite intracranial neoplasm. Peers stated "It is therefore concluded that the brain and meninges of the mouse respond only very slowly or not at all to the carcinogenic stimulus of 1:2:5:6 dibenzanthracene."

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METHOD

Twenty-one female mice of the C3H strain received intracerebral implants of pellets of 1,2,5,6-dibenzanthracene obtained from the Edcan Laboratories, South Norwalk, Connecticut. The animals were all 7 weeks of age at the start of the experiment, which was concluded when the last mouse died spontaneously. The operative procedure, anesthesia, animal care, and diet were all similar to those previously employed in experiments with methylcholanthrene (5). Pellets of the carcinogen were prepared by heating the crystals gently in a beaker until they fused; when the solidified mass cooled, cubes were cut with a knife to a diameter of 1 mm. The pellets thus obtained, without further treatment, were implanted in the right cerebral hemispheres. It should be noted that no oily or fatty vehicle, such as lard or cholesterol, was employed.

TABLE I: SURVIVAL TIME AND TYPES OF TUMORS

Mouse No.	Length of experiment, days	Tumor
1	106	Negative
2	534	"
3	106	Extracranial sarcoma
4	212	Intracerebral and extracranial sarcoma
5	342	Extracranial sarcoma
6	197	Squamous cell carcinoma of scalp
7	448	Negative
8	439	Meningeal sarcoma with extension to scalp
9	437	Extracranial sarcoma
10	266	Glioblastoma multiforme
11	297	Ependymoblastoma
13	212	Extracranial sarcoma
16	462	Negative
17	525	"
18	527	"
19	534	"
20	197	Extracranial sarcoma
23	212	" "
24	435	Negative
25	406	Extracranial sarcoma
26	414	" "

RESULTS

The pertinent data are presented in Table I. Of the 21 mice in the experiment, only 2 developed

gliomas (No. 10, glioblastoma multiforme, and No. 11, ependymoblastoma). One animal (No. 8) had an intracranial (meningeal) sarcoma and another (No. 4) had both an intra- and an extracranial sarcoma. In this animal it could not be determined where the sarcoma arose—whether intra- or extracranially or whether, indeed, it did not represent two separate tumors. Eight mice (Nos. 3, 5, 9, 13, 20, 23, 25, and 26) had extracranial sarcomas only. One animal (No. 6) developed a squamous cell carcinoma of the scalp. Tumors failed to develop in 8 mice (Nos. 1, 2, 7, 16, 17, 18, 19, and 24).

In those animals in which intracranial neoplasms did not develop, the sites of pellet implantation were marked by slit-like spaces (Fig. 1). There was neither mesodermal nor gliogenous cellular proliferation. All signs of inflammation, foreign body reaction, and phagocytosis were lacking.

Meningeal sarcoma.—Mouse 8. This animal had a diffuse neoplasm in the meninges over the right cerebral hemisphere as well as a nodular tumor extension into the underlying cortex (Fig. 2). There was also an extension of the tumor through the trephine opening in the skull with the formation of a small nodule beneath the scalp. The spindle-shaped tumor cells were arranged in parallel rows or formed whorls. Each cell had bipolar processes that combined with those of other cells to form a delicate reticular stroma. Many cells were in mitotic division and a few had more than one nucleus.

Intra- and extracranial sarcoma.—Mouse 4. The head of this mouse became progressively deformed during the last 2 weeks of life. At necropsy the deformity was found to be produced by a tumor beneath the scalp. A neoplasm also replaced much of the right cerebral hemisphere, but no apparent connection was demonstrable between this and the extracranial new growth. The cells composing the tumor were elongated and had prominent oval nuclei with much chromatin and single nucleoli. They gave rise to interlacing fibrillary processes that formed a dense stroma. Numerous mitotic figures were present as well as occasional bizarre multinucleated elements.

Glioblastoma multiforme.—Mouse 10. A large portion of the right cerebral hemisphere was replaced by a partially hemorrhagic, infiltrating tumor (Fig. 3). The neoplastic cells varied considerably in size and shape. Many were in mitotic division and some were of giant size with more than one nucleus (Fig. 5). Parts of the neoplasm were necrotic, but there was no real pseudopalisading around these zones of necrosis. The ground substance was amorphous, granular, and pale pink (in hematoxylin-eosin stain), with no definite fibrillary structure. Wilder preparations for connective tissue reticulin were negative.

Ependymoblastoma.—Mouse 11. One week before this animal died, a tumor appeared in the scalp at the site of the craniotomy. At necropsy this mass was found originating in the right cerebral hemisphere, in which was discovered the pellet of dibenzanthracene. Tumor cells infiltrated the cerebral cortex and the overlying leptomeninges. Many of them were carrot-shaped and had unipolar processes. They were often arranged in acinar formation around empty spaces or formed pseudoacini around blood vessels (Fig. 4). Intracerebral transplants of this neoplasm in other mice of the same strain yielded tumors that grew in the ventricular system and infiltrated diffusely the nervous tissue and meninges. Pseudoacini were more numerous in the transplants than in the primary tumor. In both, many cells were in mitotic division and a few were multinucleated. The primary tumor also contained several small deposits of calcium salts.

COMMENT

Four animals only in this series of 21 C3H mice developed intracranial neoplasms following intracerebral implantation of pellets of dibenzanthracene. Of these tumors 2 were gliomas and 2 sarcomas. This incidence of carcinogen-induced tumors is considerably less than half that yielded by either methylcholanthrene (5) or benzpyrene (6).

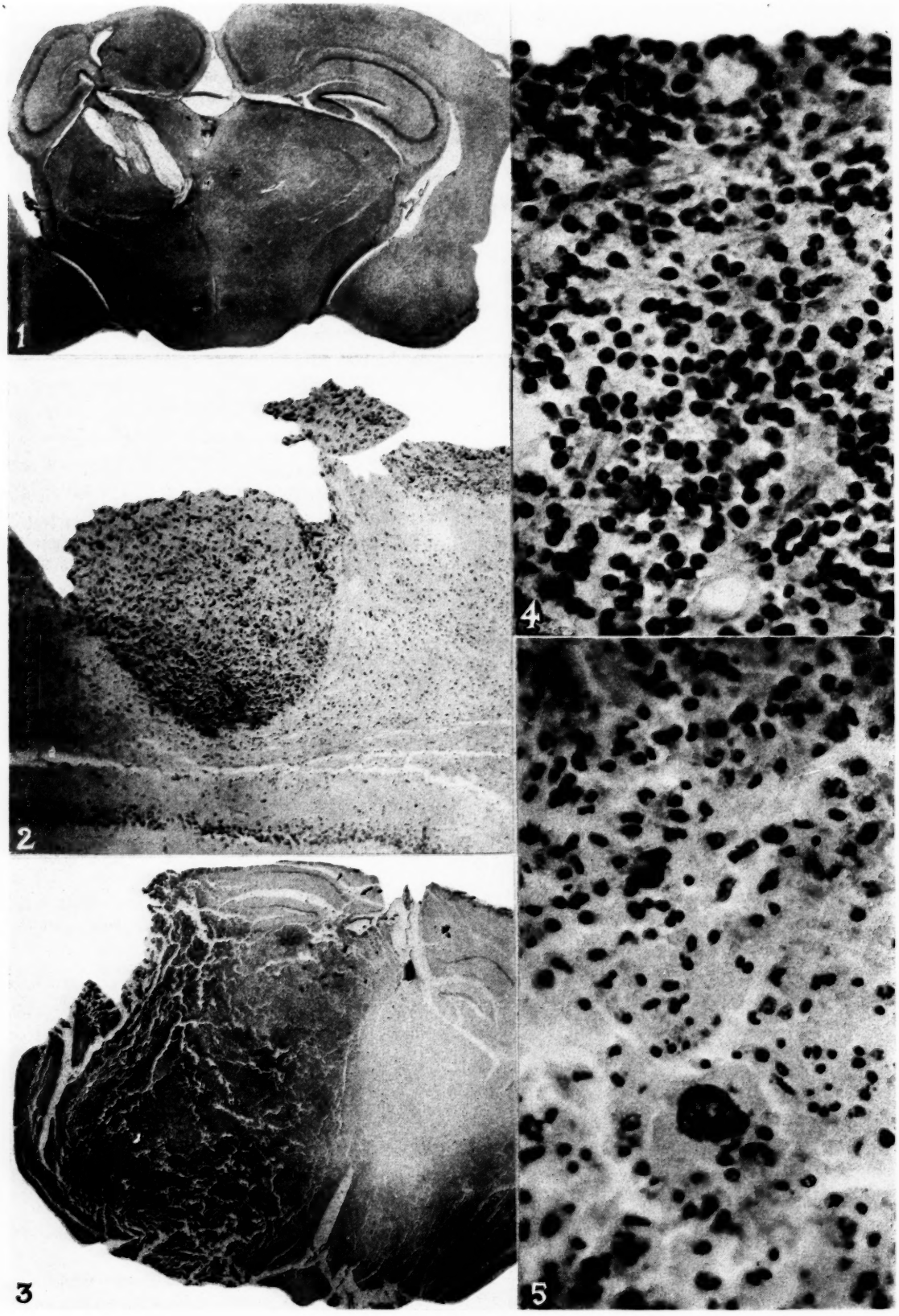
In view of the inability of Peers to induce brain tumors in stock albino mice into which were implanted cholesterol pellets containing 5 per cent dibenzanthracene, it should be emphasized that in the present experiments undiluted carcinogen was employed. This alone may account for the discrepancy in his and our results. The other difference in the two experiments was that whereas Peers employed albino mice, our animals were of the C3H strain. We have conclusive evidence (7) to show that the mouse strain is an important factor in the incidence of carcinogen-induced brain tumors.

With the single exception of the possible glioma produced by Weil with a lard solution of dibenzanthracene in a white rat, the two instances of glioma detailed in this communication constitute the first successful efforts to produce gliogenous neoplasia with this chemical carcinogen.

SUMMARY

Pellets of 1,2,5,6-dibenzanthracene were implanted in the right cerebral hemispheres of 21 female C3H mice.

Thirteen tumors developed, of which two were gliomas. One was an intracranial meningeal sarcoma. One sarcoma was both intra- and extracranial. Eight tumors were extracranial fibrosarcomas. One neoplasm



FIGS. 1–5

was a squamous cell carcinoma of the scalp. Eight animals failed to develop any type of tumor.

Of the two gliomas, one was a glioblastoma multiforme whereas the other was an ependymoblastoma.

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DESCRIPTION OF FIGURES 1 TO 5

Fig. 1.—Mouse 5. Slit-like spaces indicating site of pellet implantation in left half of interbrain of non-tumor-bearing animal. Note absence of all cellular proliferation. Mag. × 13. Specimens in Figs. 1 to 5 were stained with hematoxylin and eosin.

Fig. 2.—Mouse 8. Tumor nodule of meningeal sarcoma indenting cerebral cortex; neoplasm distinctly demarcated from nervous tissue. Meninges at right in photograph are infiltrated with tumor cells. Mag. × 30.

Fig. 3.—Mouse 10. Infiltrating tumor replacing most of right cerebral hemisphere. Mag. × 10.

Fig. 4.—Mouse 11. Tumor cells in right cerebral hemisphere showing pseudoacinar formation at top of photograph and perivascular arrangement at bottom. Mag. × 325.

Fig. 5.—Mouse 10. Pleomorphic tumor cells, some in mitotic division. Note giant tumor cell at bottom and amorphous stroma. Mag. × 325.

The Metabolism of 1,2-Benzanthracene in Mice and Rats*

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With an appendix on absorption spectra by E. R. Holiday, M. A., B. M.**

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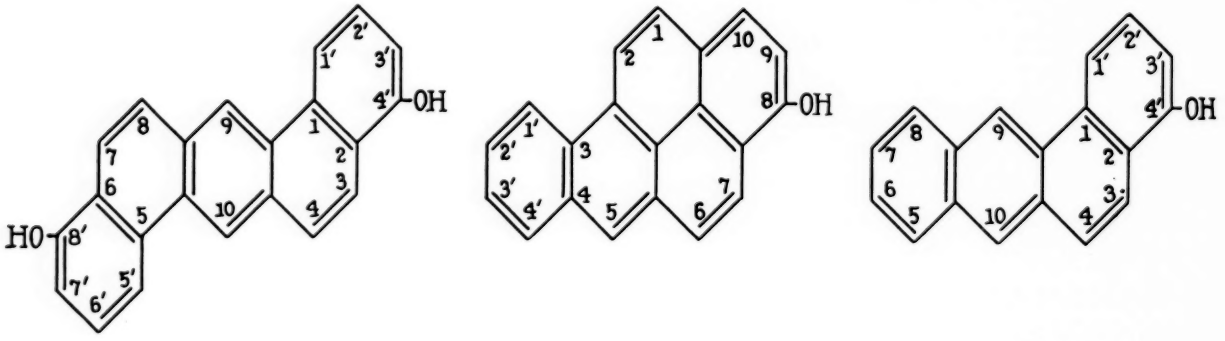
From previous metabolic studies it is known that carcinogenic hydrocarbons can be converted by the animal body into phenolic derivatives. In mice and rats, 1,2,5,6-dibenzanthracene is converted into a dihydroxy derivative (3, 8), possessing properties similar to those of synthetic 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene, formula I (4), while 3,4-benzpyrene is converted into a monohydroxy derivative (11, 5, 6, 7), which, from recent studies (2), appears to be the 8-hydroxy compound, formula II.

A similarity in the positions of the hydroxy groups in the two cases becomes apparent when the respective



I II III

parent hydrocarbons are considered as derivatives of 1,2-benzanthracene; compare formulas I, II, and III. From this it might be expected that 1,2-benzanthracene itself, when injected into mice or rats, should be converted into its 4′-hydroxy derivative, formula III. The purpose of the present investigation was to establish whether this is actually the case.

EXPERIMENTAL

The experimental procedure for the isolation of the metabolite was similar to that used previously by the authors in the study of the metabolism of 3,4-benzpyrene (1). About 2 ml. of a saturated solution of 1,2-benzanthracene in arachis oil was injected intraperitoneally per rat, and 0.4 ml. of the solution per mouse.

For 10 to 14 days after injection, the feces were collected, dried, and ground to a fine powder. This powder was extracted with cold benzene by percolation, and the pooled benzene extracts were passed through columns of alumina.[1] The columns were developed with excess of benzene, and in each case the zone showing a strong bluish white fluorescence in ultraviolet light was cut and eluted with methanol. After evaporation, the residue was methylated with dimethyl sulfate in the presence of aqueous NaOH, and the product transferred into benzene. The benzene solution was dried with anhydrous Na_2SO_4, and then passed through another column of alumina. The fluorescent filtrate, containing the methylated metabolite, was evaporated to dryness, and sublimed in high vacuum. The sublimate consisted of an oily pale yellow material, showing a tendency to crystallize.

The phenolic nature of the metabolite (*i.e.*, before methylation) was indicated by its chromatographic behavior from different solvents, and by its solubility in strong alkali, the latter being accompanied by the

* Because of the difficulties of international communication, proof of this article was not read by the authors.

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[1] "Aluminum oxide for adsorption purposes," British Drug Houses, Ltd., London.

characteristic change in fluorescence from bluish violet to yellow.

On oxidation with chromic acid it yielded a yellow product. This was not isolated, but it was found to differ from the 9,10-quinone (obtained by oxidation of benzanthracene itself with chromic acid) by its stronger adsorbability when a mixture of the two was passed through alumina. This result is compatible with the view that the oxidized yellow product of the metabolite is a hydroxyquinone and, therefore, that the OH group in the metabolite was not in the 9- or 10- position.

As in the study of the metabolism of 3,4-benzpyrene (1), conversion of the phenolic metabolite into its more stable methylated derivative proved advantageous as a practical expedient for its identification. For comparison, the following methoxy derivatives of 1,2-benzanthracene were synthesized.

4'-Methoxy-1,2-benzanthracene.—Prepared by methylation of the 4'-hydroxy compound, produced according to the method of Sempronj (12). The product was purified by chromatography and crystallization, yielding colorless needles; m.p. 160–161° C.

Analysis (Strauss and Weiler).—10.72 per cent OCH_3 (Theor. 12.02 per cent).

9,10-Dimethoxy-1,2-benzanthracene.—Prepared by reductive methylation of the 9,10-quinone, followed by purification by chromatography and crystallization. The final product consisted of colorless rhombic crystals; m.p. 137–138° C.

Analysis (Strauss and Weiler).—20.7 per cent OCH_3 (Theor. 21.5 per cent).

The 3-methoxy-1,2-benzanthracene, synthesized by Fieser and Dietz (9), was not prepared for direct comparison, as the description of its absorption spectrum by Jones (10) made it possible to establish whether or not it was identical with the methylated metabolite under investigation.

Chromatographic behavior from mixtures, fluorescence spectra, and ultraviolet absorption spectra were used as criteria for identification.

Chromatographic behavior.—Tests for identity or nonidentity of two substances can often be performed by passing the mixture of the two through chromatographic columns, and developing the columns with a suitable solvent. The resolution of the mixture into two separate zones is proof of nonidentity. When separation into two distinct zones does not occur, proof of nonidentity can still be obtained sometimes by testing successive samples of eluate from a fluid chromatogram for characteristic properties. While chromatographic resolution of a mixture is proof of nonidentity of its constituents, failure of resolution, though indicative, is not proof of identity.

Such tests were carried out with mixtures of the methylated metabolite and the synthetic compounds mentioned above. While mixtures of the methylated metabolite with the 9,10-dimethoxy compound could be resolved into the two components, no such resolution was found possible from mixtures of the methylated metabolite and synthetic 4'-methoxy-1,2-benzanthracene.

Fluorescence spectra.—This method proved to be very helpful in obtaining evidence of nonidentity between related derivatives of polycyclic hydrocarbons, and in indicating identity where the spectra were the same. In view of the relative simplicity of the technic, its high sensitivity, and its relative specificity in the presence of impurities (especially if the latter were themselves nonfluorescent), this method, together with chromatographic analysis, was used with great advantage during the preliminary stages of extraction, synthesis, or purification.

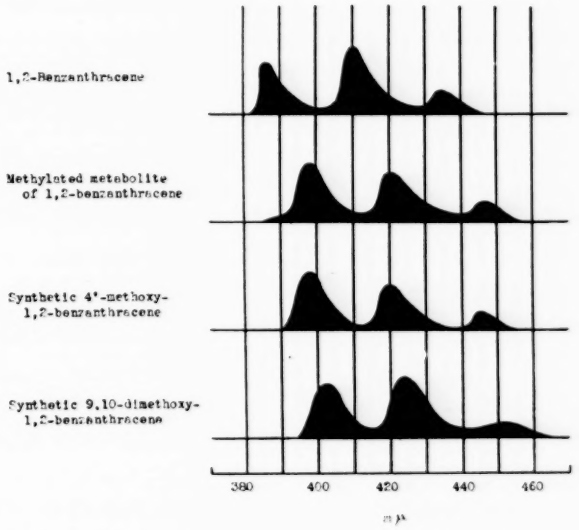

FIG. 1.—Semiquantitative representation of fluorescence spectra of 1,2-benzanthracene and some of its metabolic (methylated) and synthetic derivatives, in benzene.

Semiquantitative representations of the fluorescence spectra of benzanthracene, its 4'- and 9,10-methoxy derivatives, and the methylated metabolite, are shown in Fig. 1. It will be seen that the methylated metabolite, while differing in its fluorescence spectrum from the parent hydrocarbon and from the 9,10-dimethoxy compound, is indistinguishable from the 4'-methoxy compound.

Ultraviolet absorption spectra.—With the class of compounds under investigation (*i.e.*, those possessing multiple absorption band systems with fine structure), this method constitutes a reliable means of identification.

Comparisons were made by Dr. Holiday between the absorption spectra of the methylated metabolite and those of the other methoxy derivatives of benz-

anthracene available or described in the literature. The close agreement between the absorption spectra of the methylated metabolite and of the synthetic 4′-methoxy-1,2-benzanthracene (see appendix) suggested that they were identical.

From these results it may be inferred that the metabolite excreted in the feces was 4′-hydroxy-1,2-benzanthracene.

DISCUSSION

The results so far obtained in the studies on the metabolism of carcinogens in mice and rats appear to conform to a certain pattern. While considerably more evidence is required before the chemical mechanism involved can be understood, some tentative conclusions seem justifiable at this stage.

1. There appears to be a similarity in the positions of metabolic oxidation in the molecule as far as 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 1,2-benzanthracene are concerned; compare formulas I, II, and III.

2. The positions in the molecule metabolically attacked are not those that are most reactive chemically (the latter being the 9,10- positions in the case of benzanthracene and dibenzanthracene, and the 5- position in the case of benzpyrene). This point has already been stressed by Cason and Fieser (4).

3. It is interesting to observe that with benzanthracene and dibenzanthracene, the positions in the molecule metabolically attacked (4′- and 4′,8′- respectively) are also those where sulfonation occurs *in vitro, provided the most reactive positions (9,10-) are blocked, as in the case of quinones.*

4. These results are compatible with the view that in the process of metabolic oxidation some group, possibly an enzyme, blocks the most reactive position in the molecule, so that metabolic oxidation occurs in the *next* reactive positions.

SUMMARY

A fluorescent phenolic derivative has been isolated from the feces of mice and rats injected with 1,2-benzanthracene by the intraperitoneal route.

On methylation of the metabolite a product was obtained that possessed identical chromatographic behavior, fluorescence spectrum, and absorption spectrum (in the range longer than 300 mμ) with those of synthetic 4′-methoxy-1,2-benzanthracene.

It is suggested, therefore, that the metabolite is 4′-hydroxy-1,2-benzanthracene.

The mechanism of metabolic oxidation of carcinogenic hydrocarbons is discussed briefly.

We are indebted to Mr. F. L. Warren of the Chester Beatty Research Institute, the Royal Cancer Hospital, London, for gifts of 1,2-benzanthracene and its quinone. We wish to thank Mr. H. W. Wheal for valuable technical assistance.

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Appendix

Absorption Spectra of 1,2-Benzanthracene and of Some Methoxy Derivatives

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Preliminary observations of the ultraviolet absorption of the methylated metabolite, described in the preceding section, showed that it contained some absorbing impurities. The moving plate method previously described (1) was well suited for the investigation of this product, since the complex absorption band system shown by 1,2-benzanthracene and its derivatives is readily recognized even when contaminated by substances showing general absorption (2).

For comparison, purified 1,2-benzanthracene, 4′-methoxy-1,2-benzanthracene, and 9,10-dimethoxy-1,2-benzanthracene were available (see preceding section). The absorption curves of these three substances dissolved in hexane were measured with a Hilger-Spekker photometer and medium quartz spectrograph (flat focal plane), by means of a spark between tungsten steel electrodes. The plates were matched with a Hilger photoelectric microphotometer.

The absorption curve of 4′-methoxy-1,2-benzanthracene is plotted in Fig. 2. The position of the absorption bands of the above mentioned compounds was also determined by the moving plate method. Comparison of band positions obtained by the two methods showed close agreement, but demonstrated the greater sensitivity of the latter method in detecting inconspicuous bands. The band positions are given in Fig. 3, together with Jones' data (3) on 1,2-benzanthracene for comparison, and also his data on 3-methoxy-1,2-benzanthracene as being the only other methoxybenzanthracene of which absorption spectra are recorded in the literature. In spite of the fact that Jones used ethanol as solvent, the agreement between his results

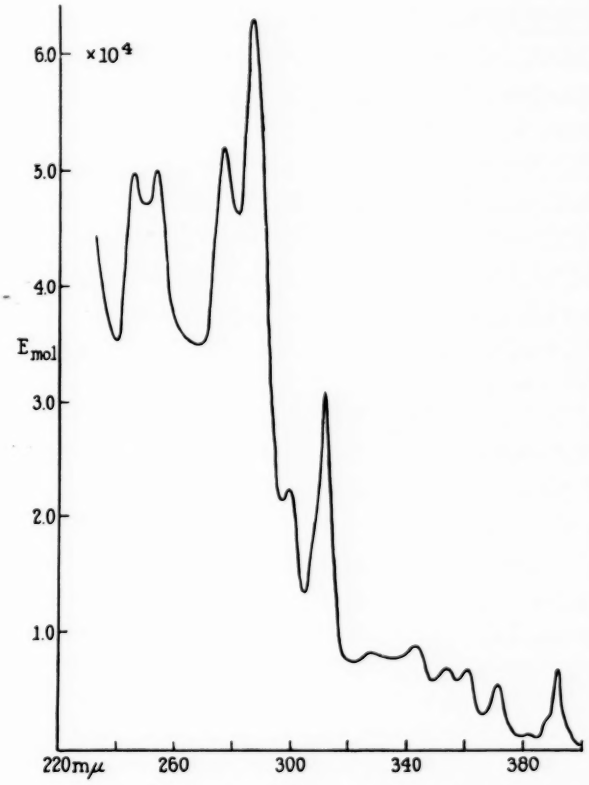

FIG. 2.—4′-Methoxy-1,2-benzanthracene in hexane.

for 1,2-benzanthracene and those determined on hexane solutions in this laboratory are very close. The extra bands in hexane are to be expected, owing to the greater degree of resolution of bands that occurs when measurements are made in solutions in hexane.

The methylated metabolite was not measured below 300 mμ, but the seven bands obtained for it agree exactly with the seven long-wave bands of 4′-methoxybenzanthracene. Moreover, the plotting of the band positions does not bring out the additional similarity that can be qualitatively observed in the relative prominence of the different bands. The 391.5 mμ band of the 4′-methoxy-1,2-benzanthracene is a very prominent one when measured in moderately concentrated solution. This feature is reproduced in the case of the methylated metabolite, as also the relative prominence of the other bands observed.

It is concluded that the evidence of the absorption spectra strongly suggests that the methylated metabolite is identical with 4′-methoxy-1,2-benzanthracene.

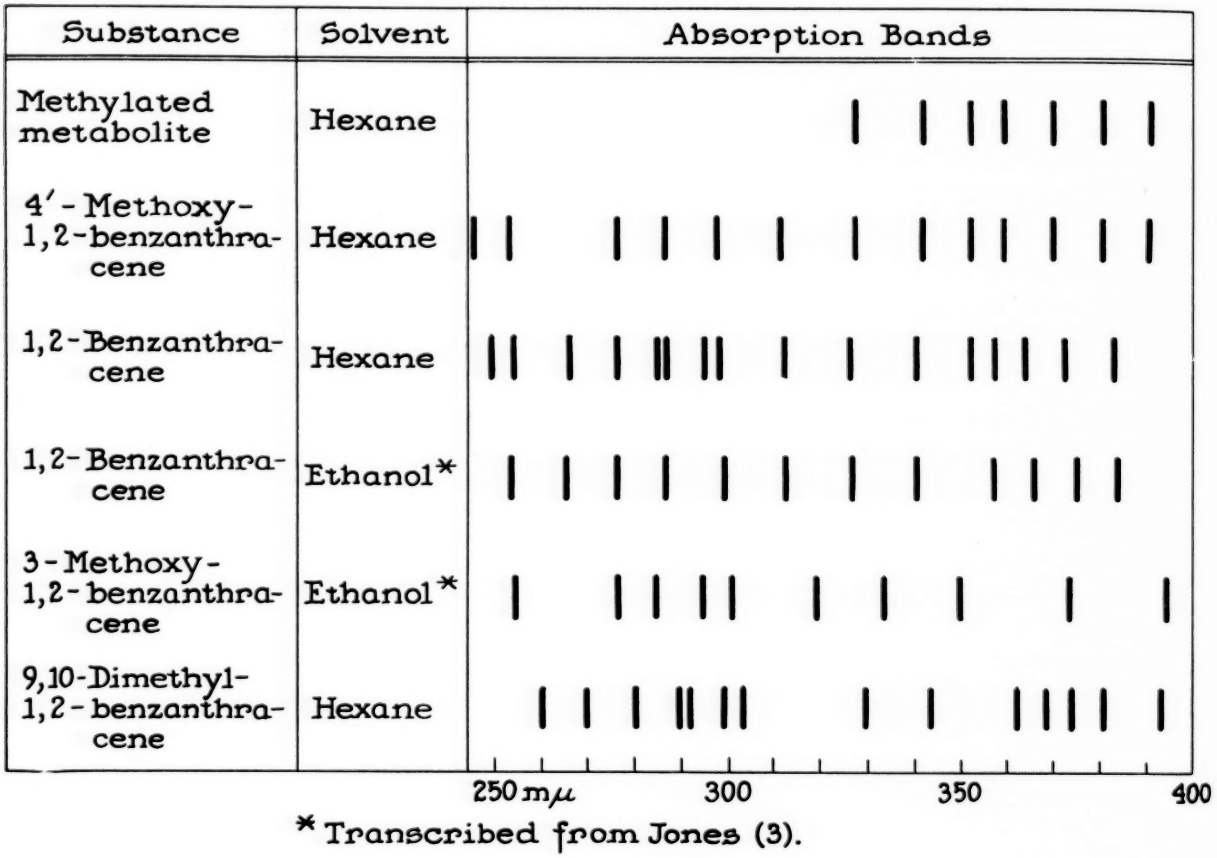

FIG. 3

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Spontaneous Primary Hepatomas in Mice of Strain C3H

II. The Influence of Breeding on Their Incidence

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Spontaneous primary tumors of the liver were observed in stock laboratory mice as early as 1908 (6). More recently these have been described in the inbred mice of strains CBA (4, 7), C3H (1, 3), C57 (5), and in a subline of C3H (2).

In most of these reports it has been shown that the neoplasms affect preponderantly the males, although Strong, Smith, and Gardner (7) reported that the tumors in the CBA strain were equally divided between the two sexes. Gorer (4), working with CBA mice over 14 months of age, reported an incidence of 48 per cent in males as compared to 7.8 per cent in females. Andervont (1) found that in strain C3H mice over one year of age 22.5 per cent of the males developed spontaneous liver tumors as compared to 11.7 per cent of the females, the majority of which were nonbreeding. In C3H mice over one year of age we (3) found the neoplasms in 53.33 per cent of breeding males as compared to none in females, the majority of which were breeding. Little, Murray, and Cloudman (5), in observations on strain C57, found 3 epithelial liver tumors in male mice, as compared to 2 in breeding females and none in nonbreeding females. In a subline of strain C3H mice over one year of age, Andervont and McEleney (2) reported primary liver tumors in 26.87 per cent of the males, as compared to 9.95 per cent of the females.

The purpose of this paper is to report further studies on hepatomas in strain C3H mice, particularly in regard to the influence of breeding on the incidence of these growths.

MATERIALS AND METHODS

The parent animals of the mice used in these experiments were obtained from the Jackson Memorial Laboratory, at Bar Harbor, Maine. The methods of breeding and caring for the mice were the same as those outlined in a previous report (3).

One hundred and thirty-three untreated mice over 12 months of age, of which 76 were males and 57 females, were subjected to postmortem examination for this study. Sixty of the males and 47 of the females were breeding, 16 of the males and 10 of the females nonbreeding.

Most of the animals were killed with chloroform when they appeared ill. The remainder died spontaneously and were examined as soon as possible after death. The presence of a liver tumor was determined by gross and microscopic examination in each instance.

TABLE I: INCIDENCE OF HEPATOMAS IN UNTREATED BREEDING AND NONBREEDING MALE AND FEMALE C3H MICE

Males						Females					
With tumors		Without tumors		Total		With tumors		Without tumors		Total	
No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.
BREEDING											
16 (27%)	13.4–21.9 Av. 15.5	44 (73%)	12.0–25.6 Av. 15.7	60 (100%)	12.0–25.6 Av. 15.6	—	—	47 (100%)	12.0–18.8 Av. 14.0	47 (100%)	12.0–18.8 Av. 14.0
NONBREEDING											
1 (6%)	— Av. 14.5	15 (94%)	12.3–20.7 Av. 17.5	16 (100%)	12.3–20.7 Av. 17.3	1 (10%)	— Av. 15.8	9 (90%)	12.2–19.8 Av. 14.2	10 (100%)	12.2–19.8 Av. 14.4

RESULTS

Sixteen (27 per cent) of the 60 untreated breeding males developed hepatomas at the average age of 15.5 months, the age range being from 13.4 to 21.9 months. The average age of the 44 animals that did not develop liver tumors was 15.7 months. The average age of all 60 breeding males was 15.6 months (Table I).

One (6 per cent) of the 16 untreated nonbreeding males developed a hepatoma at the age of 14.5 months. The average age of the 15 animals that did not was 17.5 months, the age range being from 12.3 to 20.7 months. The average age of all 16 nonbreeding males was 17.3 months (Table I).

No hepatomas were observed among the 47 untreated breeding females. The average age of the animals in this group was 14.0 months and the age range was from 12.0 to 18.8 months (Table I).

One (10 per cent) of the untreated nonbreeding females developed a hepatoma at the age of 15.8 months. The average age of the 9 animals that did not develop tumors was 14.2 months, the age range being from 12.2 to 19.8 months. The average age of all 10 nonbreeding females was 14.4 months (Table I).

COMMENT

In a previous report (3) we pointed out that spontaneous liver tumors develop more frequently in male than in female mice of strain C3H. These observations, however, were made almost exclusively on breeding mice, and we suggested that it would be necessary to study nonbreeding animals before the sex distribution of liver tumors could be determined. In the present studies the incidence of hepatomas was observed in breeding and nonbreeding animals of both sexes, an observation which, to our knowledge, has not previously been made. The division of the animals in this way, as well as the selection of animals above 12 months of age, has made some of the groups rather small, and in such groups our results must be considered as tentative. Our findings suggest, however, that there was a relatively equal incidence of liver tumors in the nonbreeding animals of both sexes, 6 per cent (1 of 16 animals) in the males and 10 per cent (1 of 10 animals) in the females. In breeding animals the incidence of hepatomas was 27 per cent (16 of 60 animals) in the males and zero (none of 47 animals) in the females. Breeding, therefore, favorably influenced the development of tumors in the males and unfavorably influenced the development of tumors in the females. There was no great difference in the average age at which the neoplasms appeared.

SUMMARY

1. The incidence of hepatomas was observed in 133 untreated breeding and nonbreeding strain C3H mice.

2. In breeding males the incidence was 27 per cent (16 of 60 animals), as compared to 6 per cent in nonbreeding males (1 of 16 animals).

3. No tumors were found in 47 breeding females, whereas 10 per cent of the 10 nonbreeding females developed them.

4. The small number of animals in some groups makes confirmatory experiments desirable.

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Spontaneous Primary Hepatomas in Mice of Strain C3H

III. The Effect of Estrogens and Testosterone Propionate on Their Incidence

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(Received for publication May 24, 1943)

Mice of various inbred strains have been shown to develop spontaneous hepatomas and attempts have been made to induce hepatomas in these animals by the administration of various agents. Strong, Smith, and Gardner (10), using strain CBA mice, were able to induce hepatomas in 8 animals receiving subcutaneous injections of 3,4,5,6-dibenzcarbazole. These neoplasms differed from the spontaneous tumors in that they appeared at an earlier age and contained bile duct epithelium. Similar treatment of strain A mice, in which no spontaneous tumors had been found, did not produce neoplasms. Andervont (1) concluded that it was uncertain whether or not liver tumors were induced in strain C3H mice receiving subcutaneous or intravenous administrations of dibenzanthracene or methylcholanthrene. The same author (2) found that mice of strain A were more susceptible than those of strain C3H to liver damage caused by *o*-aminoazotoluene (2-amino-5-azotoluene). He concluded that there was no correlation between susceptibility to the action of the dye and susceptibility to spontaneous hepatomas because the incidence of spontaneous hepatomas was higher in strain C3H than in strain A mice. Shear, Stewart, and Seligman (8) placed either dibenzanthracene, benzpyrene, or methylcholanthrene directly into the livers of mice of strains C3H, C57, and A. The incidence of hepatomas noted in strain C3H mice was within the limits of the spontaneous range and the authors concluded that the livers of these mice were refractory to the carcinogenic action of the hydrocarbons. Shimkin and Grady (9) did not observe liver lesions in strain C3H mice to which stilbestrol was administered subcutaneously or orally. Andervont and Edwards (3) were unable to produce liver tumors in mice of strains A, C, and C3H by single subcutaneous injections of 3,4,5,6-dibenzcarbazole, but the subcutaneous injection of *o*-aminoazotoluene into animals of these strains produced extensive cirrhosis and many tumors of the liver. Edwards (6) produced a high percentage of hepatomas in male mice of strain C3H and in strain A mice of both sexes by feeding a 40 per cent solution of carbon tetrachloride in olive oil. These studies were not far enough advanced to allow conclusions whether or not the tumors were directly due to the effect of the carbon tetrachloride or indrectly due to the hepatic damage. Law (7) produced hepatomas in mice of strains C57 and dba by the subcutaneous injection of four different azo compounds, the most effective of which was *o*-aminoazotoluene.

Because the incidence of spontaneous liver tumors is higher in male than in female mice of strain C3H (1, 4, 5), it occurred to us that the male sex hormone might be concerned in the development of these neoplasms, and that it might also be of interest to test the effect of estrogens on the tumor rate.

MATERIALS AND METHODS

The mice used in these experiments were of the C3H strain. The parent animals were obtained from the Jackson Memorial Laboratory, at Bar Harbor, Maine. The methods of breeding and caring for the mice were the same as those outlined in a previous report (4).

One hundred and fifty-six mice over 12 months of age were subjected to postmortem examination for this study, most of which were killed with chloroform when they appeared ill. The remainder died spontaneously and were examined as soon as possible after death.

The animals were divided into three groups as follows:

Group 1.—Forty-eight nonbreeding mice were injected subcutaneously at weekly intervals with 0.25 to 1.25 mgm. of testosterone propionate in a solution of sesame oil (perandren).[1] The injections were started at the age of 10 to 21 days and continued until death, with the exception of one group in which they were stopped after twenty-five 1.25 mgm. doses had been given. Twenty-four of these mice were males and 24

[1] We are indebted to Dr. R. MacBrayer, of the Ciba Pharmaceutical Products, Inc., for supplying us with perandren.

females. The distribution of the animals according to sex and dosage is shown in Table I.

Group II.—Sixty-nine nonbreeding mice, divided into 7 groups, were injected subcutaneously with from 400 to an average of 5,700 rat units (r.u.), 0.066 to 0.940 mgm., of α-estradiol benzoate in a solution of sesame oil (progynon B). Fifty-nine of these mice were males and 10 females. Treatment was begun at the age of 10 to 14 days and continued for periods of time varying from 3 days to an average of 57 weeks. The hormone was administered in weekly doses of 100 r.u., 0.0165 mgm., to all animals except those that received a total of 3,000 r.u., 0.498 mgm. These animals were given 2 doses of 1,500 r.u., 0.249 mgm., each on alternate days. After the course of injections was terminated in each group no further treatment was administered. The distribution of the animals according to sex and dosage is shown in Table II.

Group III.—Thirty-nine nonbreeding mice were injected subcutaneously at weekly intervals with 100 r.u. of ketohydroxyestrin in a solution of sesame oil (theelestrin)[2] beginning at ages varying from 10 days to 6 months and continuing until death. Thirty of these mice were males and 9 females. The distribution of the animals according to sex and age at the beginning of injections is shown in Table III.

Controls.—The control group consisted of 133 untreated C3H mice of which 60 were breeding and 16 nonbreeding males, and 47 were breeding and 10 nonbreeding females.

[2] We are indebted to the G. W. Carnrick Co. for supplying us with theelestrin.

TABLE I: INCIDENCE OF PRIMARY LIVER TUMORS IN NONBREEDING MALE AND FEMALE C3H MICE TREATED WITH TESTOSTERONE PROPIONATE *

	Males						Females					
	Without tumors		With tumors		Total		Without tumors		With tumors		Total	
Dose per week, mgm.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.
0.25	4 (100%)	14.7–16.9 Av. 16.3	— —	— —	4 (100%)	14.7–16.9 Av. 16.3	5 (100%)	16.9–17.1 Av. 17.0	— —	— —	5 (100%)	16.9–17.1 Av. 17.0
1.25	12 (100%)	12.1–18.3 Av. 14.6	— —	— —	12 (100%)	12.1–18.3 Av. 14.6	8 (89%)	13.7–15.4 Av. 14.1	1 (11%)	18.3	9 (100%)	13.7–18.3 Av. 14.5
1.25 (25×)	8 (100%)	14.3–16.2 Av. 14.8	— —	— —	8 (100%)	14.3–16.2 Av. 14.8	10 (100%)	12.1–16.5 Av. 15.0	— —	— —	10 (100%)	12.1–16.5 Av. 15.0
Total	24 (100%)	12.1–18.3 Av. 14.9	— —	— —	24 (100%)	12.1–18.3 Av. 14.9	23 (96%)	12.1–17.1 Av. 15.1	1 (4%)	18.3	24 (100%)	12.1–18.3 Av. 15.2

* Perandren, Ciba.

TABLE II: INCIDENCE OF PRIMARY LIVER TUMORS IN NONBREEDING MALE AND FEMALE C3H MICE TREATED WITH α-ESTRADIOL BENZOATE *

	Males						Females					
	Without tumors		With tumors		Total		Without tumors		With tumors		Total	
Dose, mgm., and rat units	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.
3,000 r.u. 0.498 mgm.	11 (69%)	12.7–21.2 Av. 17.5	5 (31%)	14.7–15.1 Av. 14.8	16 (100%)	12.7–21.2 Av. 16.6	— —	— —	— —	— —	— —	— —
400 r.u. 0.066 mgm.	6 (55%)	12.0–19.5 Av. 15.9	5 (45%)	12.4–18.8 Av. 16.1	11 (100%)	12.0–19.5 Av. 16.0	3 (100%)	12.8–13.8 Av. 13.2	— —	— —	3 (100%)	12.8–13.8 Av. 13.2
800 r.u. 0.132 mgm.	7 (100%)	13.1–21.1 Av. 16.8	— —	— —	7 (100%)	13.1–21.1 Av. 16.8	3 (100%)	14.1–15.1 Av. 14.5	— —	— —	3 (100%)	14.1–15.1 Av. 14.5
1,200 r.u. 0.198 mgm.	5 (71%)	14.7–19.4 Av. 17.3	2 (29%)	16.8–21.2 Av. 19.0	7 (100%)	14.7–21.2 Av. 17.7	1 (100%)	— Av. 12.8	— —	— —	1 (100%)	— Av. 12.8
1,600 r.u. 0.265 mgm.	7 (88%)	12.6–17.8 Av. 15.5	1 (12%)	— Av. 19.6	8 (100%)	12.6–19.6 Av. 16.0	3 (100%)	12.4–18.3 Av. 16.1	— —	— —	3 (100%)	12.4–18.3 Av. 16.1
2,000 r.u. 0.333 mgm.	6 (75%)	12.2–15.5 Av. 14.1	2 (25%)	12.2–13.6 Av. 12.9	8 (100%)	12.2–15.5 Av. 13.8	— —	— —	— —	— —	— —	— —
Av. 5,700 r.u. 0.940 mgm.	2 (100%)	12.4–14.6 Av. 13.5	— —	— —	2 (100%)	12.4–14.6 Av. 13.5	— —	— —	— —	— —	— —	— —
Total	44 (75%)	12.0–21.2 Av. 16.7	15 (25%)	12.2–21.2 Av. 15.8	59 (100%)	12.0–21.2 Av. 16.4	10 (100%)	12.4–18.3 Av. 14.5	— —	— —	10 (100%)	12.4–18.3 Av. 14.5

* Progynon-B (Schering).

The presence of a tumor was determined by gross and microscopic examination in each instance.

RESULTS

Group I.—No liver tumors developed among the 24 nonbreeding males treated with testosterone propionate. The average age of the animals in this group was 14.9 months and the age range was from 12.1 to 18.3 months (Table I).

One (4 per cent) of the 24 nonbreeding females treated with testosterone propionate developed a liver tumor at the age of 18.3 months. The average age of the 23 animals that did not develop liver tumors was 15.1 months, the age range being from 12.1 to 17.1 months (Table I).

The average age of the animals in this group was 14.5 months and the age range was from 12.4 to 18.3 months (Table II).

Group III.—Five (17 per cent) of the 30 nonbreeding male mice injected with ketohydroxyestrin developed liver tumors at the average age of 19.1 months, the age range being from 18.3 to 19.9 months. Neoplasms were found in the groups in which the injections were started at 10 to 18 days, 1 month, and 6 months respectively, and the incidence in each group was essentially the same. The average age of the 25 animals that did not develop tumors was 17.5 months, the age range being from 12.8 to 21.2 months. The average age of all 30 males was 17.7 months (Table III).

TABLE III: INCIDENCE OF PRIMARY LIVER TUMORS IN NONBREEDING MALE AND FEMALE C3H MICE TREATED WITH KETOHYDROXYESTRIN *

	Males						Females					
	Without tumors		With tumors		Total		Without tumors		With tumors		Total	
Age at start	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.	No. of mice	Age range and av. age, mos.
10–18 days	6 (75%)	13.1–19.9 Av. 17.7	2 (25%)	Av. 19.9	8 (100%)	13.1–19.9 Av. 18.2	3 (100%)	13.4–16.0 Av. 14.3	—	—	3 (100%)	13.4–16.0 Av. 14.3
1 month	2 (67%)	17.0–18.3 Av. 17.6	1 (33%)	Av. 18.3	3 (100%)	17.0–18.3 Av. 17.8	—	—	—	—	—	—
2 "	1 (100%)	— Av. 13.2	—	—	1 (100%)	— Av. 13.2	3 (100%)	12.6–13.7 Av. 13.0	—	—	3 (100%)	12.6–13.7 Av. 13.0
3 "	3 (100%)	12.8–13.6 Av. 13.3	—	—	3 (100%)	12.8–13.6 Av. 13.3	—	—	—	—	—	—
4 "	1 (100%)	— Av. 21.6	—	—	1 (100%)	— Av. 21.6	—	—	—	—	—	—
5 "	7 (100%)	13.1–19.8 Av. 17.3	—	—	7 (100%)	13.1–19.8 Av. 17.3	1 (100%)	— Av. 14.8	—	—	1 (100%)	— Av. 14.8
6 "	5 (71%)	18.0–19.8 Av. 19.3	2 (29%)	19.6–19.8 Av. 19.7	7 (100%)	18.0–19.8 Av. 19.4	2 (100%)	12.0–12.4 Av. 12.2	—	—	2 (100%)	12.0–12.4 Av. 12.2
Total	25 (83%)	12.8–21.2 Av. 17.5	5 (17%)	18.3–19.9 Av. 19.1	30 (100%)	12.8–21.2 Av. 17.7	9 (100%)	12.0–16.0 Av. 13.5	—	—	9 (100%)	12.0–16.0 Av. 13.5

* Theelestrin, Carnrick.

Group II.—Fifteen (25 per cent) of the 59 nonbreeding males injected with α-estradiol benzoate developed liver tumors at the average age of 15.8 months, the age range being from 12.2 to 21.2 months. The 11 animals that received 400 r.u., 0.066 mgm., showed the highest incidence, 45 per cent. The next highest incidence, 31 per cent, was observed in the 16 animals that received 3,000 r.u., 0.498 mgm. The incidence in the remaining groups varied from 29 per cent in the 7 animals receiving 1,200 r.u., 0.198 mgm., to zero in the 7 animals receiving 800 r.u., 0.132 mgm., and in the 2 animals receiving an average of 5,700 r.u., 0.940 mgm. The average age and age range for these various groups is shown in Table II. The average age of the 44 treated males that did not develop tumors was 16.7 months, the age range being from 12.0 to 21.2 months.

No liver tumors were observed among the 10 nonbreeding female mice treated with α-estradiol benzoate.

No liver tumors developed among the 9 nonbreeding female mice treated with ketohydroxyestrin. The average age of the animals in this group was 13.5 months and the age range was from 12.0 to 16.0 months (Table III).

Control group.—The incidence of hepatomas was 27 per cent in breeding males, 6 per cent in nonbreeding males, zero in breeding females, and 10 per cent in nonbreeding females. The age range and average age have been given in the preceding report (5).

DISCUSSION

No tumors developed among the 24 nonbreeding male animals treated with testosterone propionate and only one of 24 treated female mice showed a neoplasm. In the treated males the incidence of liver tumors was lower than that seen in either breeding or nonbreeding male controls. In the females the incidence (4 per cent) in the treated animals was approximately

midway between the incidence in breeding and nonbreeding controls, zero and 10 per cent respectively. The age at which the single tumor appeared in the treated female was considerably above the average age at which spontaneous tumors appeared in the controls.

The average incidence of liver tumors, 22 per cent, in the 89 nonbreeding males treated with either *a*-estradiol benzoate or ketohydroxyestrin was higher than that, 6 per cent, of the 16 untreated nonbreeding males. Although the number of mice was too small for definite conclusions, in some groups the incidence was higher than that of the 60 untreated breeding males. The highest incidence, 45 per cent (5 of 11 mice), seen after the injection of 400 r.u., 0.066 mgm., of *a*-estradiol benzoate, was well above the incidence, 27 per cent (16 of 60 mice), seen in untreated breeding control males. The average age at which tumors developed in the treated animals was somewhat above that seen in control animals. No liver tumors appeared in the 19 nonbreeding females treated with either *a*-estradiol benzoate or ketohydroxyestrin.

These findings suggest that the effect of estrogen injections in the 89 nonbreeding males was to elevate the incidence of tumors not only above that observed in the 16 untreated nonbreeding males, but even in some cases above that observed in the 60 untreated breeding males. It is likewise suggestive that the effect of estrogen injections in the 19 nonbreeding females was to depress the incidence of tumors to the zero level found in the 47 untreated breeding females. However, the evaluation of the effect of estrogen treatment on the incidence of liver tumors must be considered as tentative, because the number of mice in many groups was small. Although large numbers of animals were started on estrogen injections, many died of mammary gland carcinoma and urinary tract calculi before they attained the age at which liver tumors develop, a circumstance that made only small numbers of mice available for study.

SUMMARY

1. Forty-eight nonbreeding C3H mice were treated with testosterone propionate, 69 nonbreeding C3H mice with *a*-estradiol benzoate, and 39 nonbre C3H mice with ketohydroxyestrin.

2. None of the 24 males and 4 per cent of th females injected with testosterone propionate devel hepatomas.

3. Twenty-five per cent of the 59 males and n of the 10 females injected with *a*-estradiol benz developed hepatomas.

4. Seventeen per cent of the 25 males and n of the 9 females injected with ketohydroxye developed hepatomas.

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ontaneous Primary Hepatomas in Mice of Strain C3H

V. A Study of Intracytoplasmic Inclusion Bodies and Mitochondria

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describing the histology of spontaneous hepatomas train C3H mice, we pointed out (2) that the plasm of the tumor cells contained two types of usion bodies. One of these was a large, homo- eous, or finely granular, hyaline body that often hed the nucleus to one side; the other a rounded, ometimes indented, pink-staining body that varied tly in size and sometimes showed a doubly refrac- ring at the periphery.

Other investigators also have found cell inclusions hepatomas. Edwards and Dalton (4) described ular inclusions in the cytoplasm of cells of a pri- y liver neoplasm of a strain C3H mouse, as well in the first generation transplant of this tumor. wards and White (5) described cytoplasmic inclu- s in hepatomas produced in rats by feeding methylaminoazobenzene. These inclusions varied ze, the larger ones being somewhat larger than verage nucleus of the hepatoma cells. They were acid-fast and usually were acidophilic. When ed with Mallory's aniline blue stain they showed ntral blue core surrounded by an unstained zone side of which was a blue marginal rim. The in- sions were sometimes found free in spaces that re possibly lymph vessels.

The purpose of this paper is to report further lies on the various types of inclusion bodies found ne cells of hepatomas arising in strain C3H mice.

MATERIALS AND METHODS

The 88 mice used in these experiments were of the H strain. The source of the animals and the methods of breeding and caring for them have been tlined in a previous report (2).

Thirty-nine of the mice had hepatomas. Eighteen were untreated control mice; of these 16 were breeding males, 1 was a nonbreeding male, and 1 a nonbreeding female. Twenty-one were treated animals; of these 15 were nonbreeding males treated with α-estradiol benzoate; 5 were nonbreeding males treated with ketohydroxyestrin, and 1 was a nonbreeding female treated with testosterone propionate. Details as to dosage and time of injection have been given in a previous report (6).

Forty-nine of the mice did not have liver tumors. Of these 16 were untreated controls, of which 7 were breeding and 6 nonbreeding males and 1 was a breeding and 2 were nonbreeding females. Thirty-three were treated animals. Three nonbreeding males and 1 nonbreeding female were treated with α-estradiol benzoate; 10 nonbreeding males and 1 nonbreeding female were treated with ketohydroxyestrin; 9 nonbreeding males and 9 nonbreeding females were treated with testosterone propionate.

Most of the animals were killed with chloroform when they appeared ill. The remainder died spontaneously and were examined as soon as possible after death. Pieces of liver were fixed in Helly's fluid and in 10 per cent formalin. Microscopic sections, stained with hematoxylin and eosin, and with Mallory's phosphotungstic acid–hematoxylin stain for mitochondria, were prepared on hepatoma and liver tissue from all animals. In addition, sections of many, but not all, hepatomas and livers were stained with Mallory's aniline blue stain for connective tissue, Van Gieson's stain for connective tissue, Mallory's phloxine and methylene blue, Mallory's stain for alcoholic hyalin, Ziehl-Neelsen's carbolfuchsin, methyl violet, acidulated potassium ferrocyanide, and sudan IV.

RESULTS

Macroscopic studies.—The gross appearance of the tumors was the same as that described in a previous report (2). In general the neoplasms were solid, rounded, well localized, but not encapsulated masses. When located within the liver, they were gray; the rarer pedunculated tumors were sometimes hemorrhagic. Metastases were not observed.

Microscopic studies.—On the whole the tumor cells closely resembled normal liver cells and arranged themselves in cords. Accurate reproduction of liver lobules did not occur and, although bile canaliculi were observed regularly, bile ducts were rarely, if ever, present.

The cytoplasm and nuclei of the liver and tumor

cells contained various bodies that were made the subject of special study. Some were mitochondria. The others were cellular inclusions, of which we were able to identify two types: (a) intracytoplasmic hyaline bodies, and (b) intracytoplasmic lipoprotein bodies.

Mitochondria.—The presence of numerous granules of varying size, shape, and staining qualities in the neoplastic and nonneoplastic liver cells makes it necessary to define our interpretation of mitochondria. With Mallory's phosphotungstic acid–hematoxylin stain for mitochondria after fixation in Helly's fluid, only the small coccoid or rod-like, sharply defined bodies that stained deep blue were accepted as mitochondria (Fig. 1).

In the livers of untreated control animals, mitochondria were constantly present. The cells containing them were irregularly distributed in small to large patches. In the livers of animals treated with testosterone propionate the mitochondria were not reduced in numbers, but in those that received estrogens the mitochondria were either definitely reduced in numbers or completely absent. In the hepatomas the mitochondria were usually sparse or absent. Occasionally they occurred in very large numbers throughout the tumor. One necrotic tumor contained numerous mitochondria.

Intracytoplasmic hyaline inclusion bodies.—These consisted of homogeneous or very finely granular masses that measured 10 to 15 microns in diameter and were confined to the cytoplasm. They were present in the majority of the hepatomas. The only nonneoplastic liver tissue that contained these bodies was that at the periphery of one tumor. With hematoxylin and eosin these bodies stained pink and with Mallory's phosphotungstic acid–hematoxylin, pale blue. A few contained small, rounded, pale staining bodies that resembled lipoprotein bodies.

Intracytoplasmic lipoprotein inclusion bodies.—These bodies were found exclusively in the cytoplasm of the neoplastic cells and were noted in every tumor. The total number in each tumor varied from a few to innumerable bodies. The size of the growth and the presence of necrosis bore no demonstrable relationship to the number present. In the tumors that had undergone complete coagulation necrosis the number of visible bodies was diminished, and faint, rounded "ghost-like" bodies suggested that some had undergone degeneration. The distribution within the neoplasms was highly irregular. The location of the bodies within the tumor bore no relationship either to the central or peripheral portions of the growth, or to the blood vessels. No one portion of the cytoplasm was more frequently the site of these inclusions than any other. The number contained within a single cytoplasm usually varied from 1 to 50; occasionally there were more. The cells containing these inclusions often appeared little altered but in some cases, especially when the inclusion bodies were numerous or large, the cell nucleus was pyknotic or absent and the cell membrane ruptured.

The bodies varied from 3 to 13 microns in diameter, the smaller ones being the most numerous (Fig. 2). The majority appeared to be spheroidal, but many were ovoid and others appeared as indented spheroids or crescent-shaped bodies. Most of the smaller bodies appeared to be homogeneous. Many of the medium sized and larger ones, however, contained pale, rounded or ovoid masses that in rare instances presented protrusions resembling those seen in budding yeast cells (Fig. 3). Other bodies contained either a brownish bur-like mass or scattered brownish granules.

In sections stained with hematoxylin and eosin most of the lipoprotein bodies were strongly eosinophilic. Others showed unstained or pale green doubly refractile peripheral rims and a central eosinophilic granular or nongranular mass or masses (Fig. 3). In some tumors many of the bodies were unstained; others were pale pink or light green.

With the phosphotungstic acid–hematoxylin stain (Fig. 4) most of the bodies stained deep blue or reddish blue though a few had a reddish brown color or a distinct greenish tint. Among the medium sized and larger bodies some had a deep blue peripheral rim and a central mass, or masses, that stained a paler blue.

With phloxine–methylene blue most of the bodies stained deep red or purplish red, although some of them were stained pale pink and others were mottled with red and blue stain.

With Mallory's aniline blue connective tissue stain the bodies stained orange or reddish orange, with the peripheral rim often staining more deeply than the central portion.

With Van Gieson's connective tissue stain they stained yellow, with the peripheral rims sometimes colored brownish yellow.

Mallory's phloxine stain for alcoholic hyalin showed that the central portions of a few bodies stained pink.

Ziehl-Neelsen's carbolfuchsin stain showed that many of the bodies, both large and small, were acid-fast. The peripheral rim was stained more deeply in some cases.

Frozen sections of formalin-fixed tumor tissue, stained with sudan IV, showed that almost all the bodies stained intensely red. In some of the bodies the peripheral rims stained more deeply. In tissues that had been dehydrated with alcohol, infiltrated with chloroform, embedded in paraffin, and treated with xylol, many of the bodies had a reddish orange color

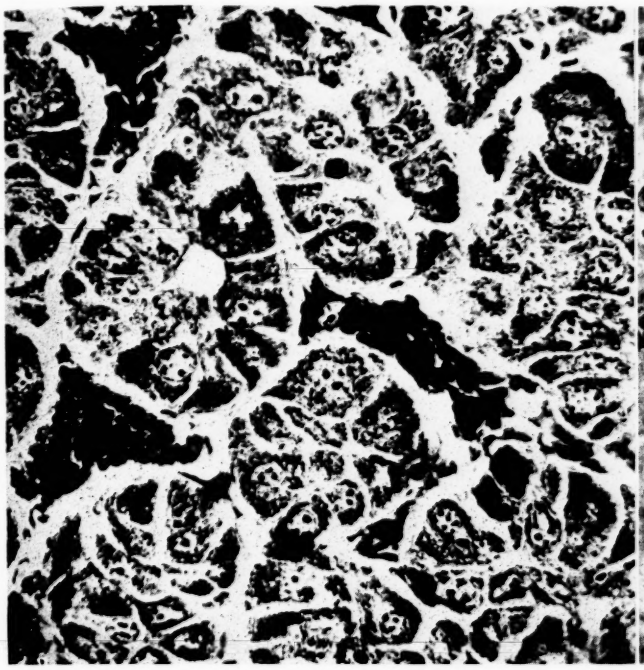

FIG. 1.—Mitochondria in liver cells of an untreated male mouse 17.6 months of age. Small, sharply staining coccoid or rod-like bodies in the cytoplasm. Mallory's phosphotungstic acid–hematoxylin stain for mitochondria. Mag. × 480.

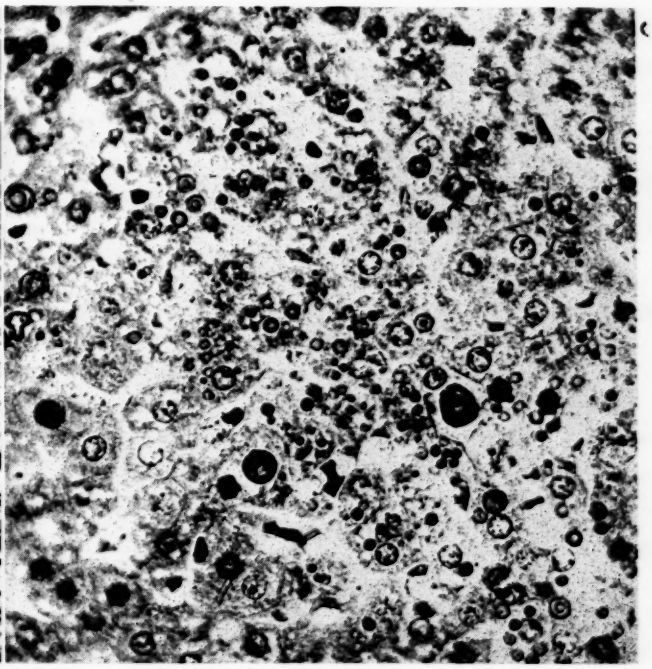

FIG. 2.—Lipoprotein bodies in hepatoma cells of an untreated male mouse 14.5 months of age. There is great variation in the size of these rounded bodies and in the number contained in the cytoplasm of the hepatoma cells. Doubly refractile rims at the margins of some inclusions. Mallory's phloxine and methylene blue stain. Mag. × 480.

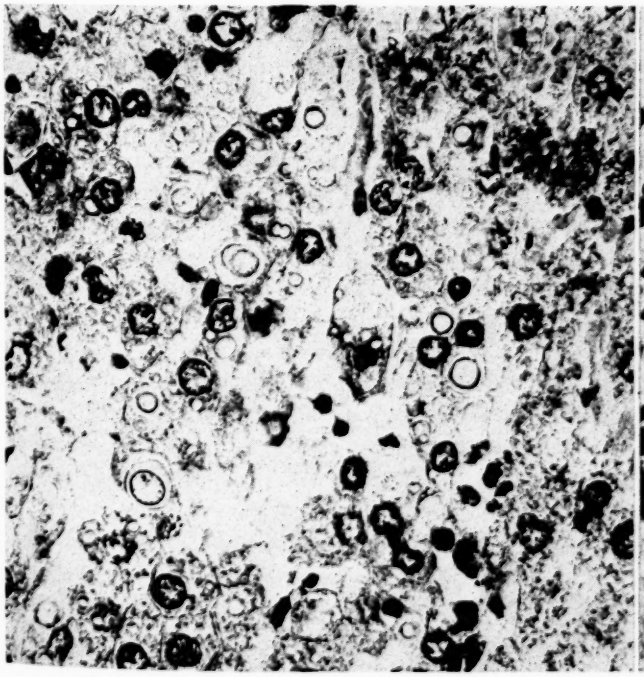

FIG. 3.—Lipoprotein bodies in hepatoma cells of an untreated male mouse 16.4 months of age. The larger bodies show doubly refractile rims and central granular or nongranular masses. Bud-like protrusions of two masses. Hematoxylin and eosin stain. Mag. × 480.

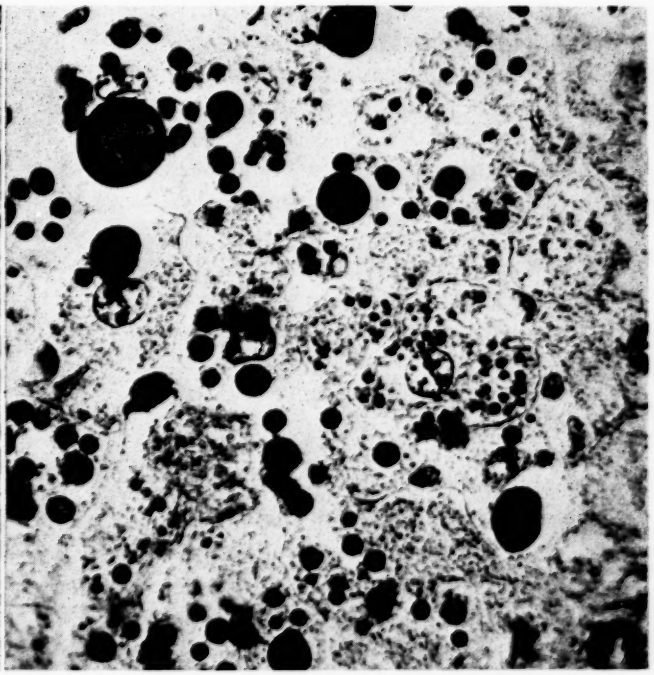

FIG. 4.—Lipoprotein bodies in hepatoma cells of an untreated male mouse 13.5 months of age. The bodies vary greatly in size and stain deeply. Some show a densely stained peripheral rim and a lighter central area. Mallory's phosphotungstic acid-hematoxylin stain for mitochondria. Mag. × 480.

with the sudan IV stain. Most of those that stained were medium to small in size.

The application of acidulated potassium ferrocyanide revealed that some of the bodies contained minute blue-staining granules. The inclusions did not stain positively either for hemofuscin with Mallory's fuchsin stain, or for amyloid with methyl violet.

COMMENT

Of the various intracellular bodies, the mitochondria and the lipoprotein inclusion bodies appeared to be of the greatest interest. It must be pointed out that our methods of studying mitochondria do not fulfill the requirements for accurate assay. Such studies have recently been made by Dalton and Edwards (3). These authors found only filamentous forms of mitochondria in the cells of induced hepatomas and predominantly spherical forms in spontaneous hepatoma cells. Normal liver cells contained filamentous, spherical, and short rod-like forms. These authors do not make statements concerning the relative numbers of mitochondria found in normal and neoplastic liver, and consequently we feel that our data, although of limited accuracy, may be of some value in this respect. In our material mitochondria were distributed in the cytoplasm of the liver cells of all the untreated mice, although the number and distribution of these organoids was variable. In general, mitochondria were not numerous in the liver tumors of any group, regardless of the treatment given, although some of the tumors that did show them presented unusually large numbers. The administration of testosterone propionate did not notably influence the number or distribution of the mitochondria, but after the administration of estrogens the numbers were reduced until, in many cases, none could be found. We do not feel that our studies permit us to state whether or not there is any correlation between this reduction in the number of mitochondria and the appearance of tumors in the estrogen-treated animals on the one hand, and the unaltered numbers of mitochondria and the absence of liver tumors in the testosterone-treated animals on the other hand. It is assumed that many mitochondria were lost from our tissues in the processes of dehydration and embedding, since our tissues were not treated with chromate after fixation. This might explain the variation in the number of mitochondria in the livers of untreated animals. It would not explain the consistently large difference in number found between the estrogen- and testosterone-treated groups.

The lipoprotein inclusion bodies were peculiar to the cytoplasm of neoplastic liver cells of all tumors. The term lipoprotein body has been given to these structures because a lipid and a protein content was suggested by their staining reactions and solubilities. Their lipid content was demonstrated by the use of sudan IV. That the bodies continued to stain with sudan IV, even after paraffin embedding, indicated that they were not composed entirely of neutral fats, but contained, in addition, some complex lipid substance that was not soluble in chloroform, alcohol, or xylol. This view was supported by the acid-fast character of these bodies after treatment with carbolfuchsin. The protein content was shown by the staining reactions with many of the other stains used. We have adopted the term lipoprotein body largely for purposes of convenience in describing these structures, and we do not wish to imply that other substances are not present. Additional studies are necessary to establish their exact composition.

We do not know the origin or significance of the lipoprotein bodies. Several possibilities present themselves for consideration. It is possible that they originated from the large hyaline intracytoplasmic bodies that were found almost exclusively in the tumor cells. This was suggested by the presence of small bodies, resembling lipoprotein bodies, in the substance of some of these inclusions. The proportionately small number of these hyaline bodies and the infrequency with which possible transitions from one type to the other was observed, speaks against this view.

It is likewise possible that these bodies arose from mitochondria, or that there was some relationship between the reduced number of mitochondria and the formation of the lipoprotein bodies in the neoplasms. Both stained similarly with phosphotungstic acid–hematoxylin, and in one case numerous mitochondria and small lipoprotein bodies were present in the same tumor. Moreover, according to some authorities (1), mitochondria contain a complex lipid substance. In the smallest tumor (0.1 cm.) that we have yet observed, however, the lipoprotein bodies were already numerous and the mitochondria scarce; there was no evidence of transition from one to the other. In addition, although the mitochondria were greatly reduced in numbers in the nonneoplastic portions of the livers of the estrogen-treated animals, no lipoprotein bodies were found. We do not feel that the mitochondria are a likely source of these bodies.

The doubly refractile rim at the periphery of the lipoprotein body, the content of granular material, as well as of single or multiple pale staining bodies, suggests that these bodies may be yeast-like organisms or animal parasites. We know of no parasites, however, that these bodies resemble, and inoculations of culture media and injections of animals with material from the tumors have failed to demonstrate an infectious agent (2).

That these bodies may result from products of cell

degeneration *per se,* like, for example, those seen in hyaline degeneration and fatty metamorphosis, seems unlikely because the bodies were often present in great numbers in tumors where degenerative changes were completely absent.

Finally, it is possible that these bodies represent altered products of cell secretion. This hypothesis receives some support from the fact that the centers of some contained a brown, granular, iron-containing material that may have been a by-product in the formation of bile. Retention of secretory products, however, did not seem to play an important role in the formation of these bodies because the bile capillaries were not distended with secretion.

SUMMARY

1. Intracytoplasmic inclusion bodies and mitochondria were studied in the liver and hepatoma cells of strain C3H mice.

2. Two types of intracytoplasmic inclusion bodies were found. One was a large hyaline body discovered almost exclusively in the cytoplasm of tumor cells, the other a smaller, lipoprotein body found exclusively in the cytoplasm of the tumor cells. The staining reactions of these bodies are described and their possible modes of origin discussed.

3. Mitochondrial studies were made under conditions that did not permit accurate assay of their numbers or forms. Gross comparative studies, however, showed that: (a) There were few mitochondria in hepatoma cells as compared to nonneoplastic liver cells. (b) The administration of testosterone propionate did not alter the number of mitochondria in nonneoplastic liver cells, and the administration of *a*-estradiol benzoate reduced the number of mitochondria in nonneoplastic liver cells, as compared to nonneoplastic liver cells of untreated animals.

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Tumor Inhibitor Studies

II. The Effect of Plant Hormones on Tumor Growth*

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In a recent preliminary search for chemicals that might influence neoplastic growth, a large number of compounds were tested. The most pronounced effects were observed with 3-indolebutyric acid and 3-indolepropionic acid, two potent plant hormones that, at relatively low levels, successfully prevented takes of the Flexner-Jobling rat carcinoma (2). Potassium α-naphthaleneacetate and 3-indoleacetic acid, two other plant hormones, have also been reported to retard the growth rate of the Bashford mouse carcinoma and of a transplantable hepatoma of rats (3, 4). Accordingly, 23 plant hormones were tested for their effect on tumor growth.

METHODS

Both young adult albino ABC mice and Sprague-Dawley rats weighing from 60 to 80 gm. were used in this study. The mice were kept in groups of 20 and the rats in groups of 10 with Purina dog food and water available at all times. The three tumors used were the Flexner-Jobling rat carcinoma, the Walker rat carcinosarcoma 256, and a transplantable fibrosarcoma obtained from the ear of a mouse that had been subjected to prolonged ultraviolet irradiation; all grew in from 90 to 95 per cent of the inoculated animals. The effect of each compound was studied on at least two and sometimes on all three of the tumors. Two methods of testing were employed for all the compounds. The first, previously described (2), was used only with the rat tumors. Vigorously growing Flexner-Jobling or Walker 256 tumors were removed aseptically, cleaned of any extraneous or necrotic tissue, and forced through a small mincer having a plate 2 cm. in diameter with holes approximately 0.6 mm. in diameter. The mince was suspended in 2 volumes of 0.8 per cent sterile sodium chloride solution, and 0.5 cc. of this was then thoroughly mixed with the same volume of a test solution by drawing back and forth in a syringe. The final concentration of the test solution for each compound is listed in Table I. As a control, the same procedure was used except that the tumor suspension was mixed with an equal volume of saline solution instead of the test solution. It was necessary to have the number of suspended cells as uniform as possible in all tests since, with any given concentration of inhibitor solution, less inhibition was observed with heavy tumor suspensions than with dilute ones. After these suspensions had stood for 30 minutes at room temperature, rats were inoculated subcutaneously with 0.2 cc. of the untreated (control) tumor in the right groin and with 0.2 cc. of a treated suspension in the left groin. An 18 gauge needle was used for the inoculations. Movement of the syringe before each injection maintained the suspension and ensured approximately the same number of cells in each inoculation. Five rats were used for each test, and all positive or questionable results were repeated. Tumor growth was followed for 3 weeks with measurements at weekly intervals. Inhibition was considered "good" if the treated tumor was less than one-fourth the size of the control, and "partial" if no tumor arising from the treated material was more than one-half the size of the control. No inhibition was considered significant unless the difference in size were evident in every animal of the group. If the results did not conform to the criteria specified above they were classed as negative. Thus mild or irregular responses were ignored.

In the second method of testing, mice and rats bearing the fibrosarcoma or the Flexner-Jobling tumor respectively were given subcutaneous injections of aqueous or oily solutions or suspensions of the chemicals once or twice daily at a site distant from the tumor. The treatment was started 2 days after the tumor inoculation and continued for 16 to 20 days. In general, the amounts tested for tumor inhibition were considerably less than the toxic levels. Five animals were used for each test, and tumor growth was measured at weekly intervals for 3 weeks.

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TABLE I: EFFECT OF PLANT HORMONES ON THE TAKES AND GROWTH OF THE FLEXNER-JOBLING RAT CARCINOMA

Tumor Mince in Direct Contact with a Solution of the Chemical Prior to Inoculation

Chemical	Amount used, mgm. per cc.	Resulting inhibition
α-Naphthaleneacetic acid *	0.5	Good
3-Indolebutyric acid	2.5	Good
	1.75	Good
	0.5	Good
	0.25	Partial
	0.05	0
Sodium ω-1-naphthoxyvalerate	0.5	Good
	0.25	Partial
Sodium ω-2-naphthoxyvalerate	0.5	Good
Sodium ω-2-naphthoxyheptanoate	0.5	Good
	0.25	Partial
Sodium S-(1-naphthyl)thioglycolate	0.5	Partial
3-Indoleacetic acid	0.5	Partial
Sodium 2-naphthoxyacetate	0.75	Partial
Sodium 2-(4-chlorobenzoyl)benzoate	2.5	Good
	1.75	Good
Sodium β-1-naphthoylpropionate *	5.0	Good
	2.5	Partial
	0.5	0
Sodium 1-naphthoxyacetate *	5.0	Good
	2.5	Partial
	0.5	0
Sodium β-1-naphthoxypropionate	5.0	Good
	2.5	Partial
Disodium 2-dodecene-1,10-dicarboxylate	7.5	Good
	5.0	Partial
Sodium β-2-naphthoylpropionate	7.5	Good
	5.0	Partial
Sodium 1-naphthylmethanesulfonate	7.5	Good
	5.0	Partial
Sodium β-naphthylhomophthalimide	7.5	Good
	5.0	Partial
Sodium *p*-chlorophenoxyacetate	7.5	Good
	5.0	Partial
3-Indolepropionic acid	10.0	Good
	5.0	Partial
	2.5	Partial
	0.5	0
Disodium 1,4-dihydro-1,4-naphthalenedicarboxylate	5.0	0
Sodium *o*-chlorophenoxyacetate	5.0	0
Sodium γ-1-naphthylbutyrate	5.0	0

* Walker carcinosarcoma 256 also used.

RESULTS AND DISCUSSION

When tumor mince obtained from rats was allowed to stand in direct contact with the plant hormones preceding inoculation, a definite retardation of takes or of subsequent growth of cells was generally observed (Table I). α-Naphthaleneacetic acid, 3-indolebutyric acid, sodium ω-1-naphthoxyvalerate, sodium ω-2-naphthoxyvalerate, and sodium ω-2-naphthoxyheptanoate were the most effective of the compounds tested, all causing definite inhibition when concentrations of 0.5 mgm. per cc. were employed. Sodium S-(1-naphthyl)-thioglycolate and 3-indoleacetic acid gave partial inhibition at 0.5 mgm. per cc. Sodium 2-naphthoxyacetate and sodium 2-(4-chlorobenzoyl)benzoate retarded tumor takes when solutions containing 0.75 to 1.75 mgm. per cc. were used. Sodium β-1-naphthoylpropionate, sodium 1-naphthoxyacetate, sodium β-1-naphthoxypropionate, disodium 2-dodecene-1,10-dicarboxylate,[1] sodium β-2-naphthoylpropionate, sodium 1-naphthylmethanesulfonate, sodium β-naphthylhomophthalimide, sodium *p*-chlorophenoxyacetate, and 3-indolepropionic acid were all active when employed at levels of 5 to 10 mgm. per cc. The plant hormones disodium-1,4-dihydro-1,4-naphthalenedicarboxylate, sodium *o*-chlorophenoxyacetate, and sodium γ-1-naphthylbutyrate were inactive at the concentrations used, and N-(*p*-chlorophenyl)glycine hydrochloride, β-1-naphthylethanol and β-1-naphthoxyethanol could not be tested by this method because of their insolubility in water. Minimum effective doses were not determined for all the agents, and it is possible that some might have been effective in lower amounts. The minimum dose for 3-indolebutyric acid appeared to be 0.25 mgm. per cc., at which concentration partial inhibition resulted. In all these experiments the animals remained in good health and the control tumors grew well.

The effect of these compounds on tumor growth was tested also by subcutaneous injection into animals bearing well established neoplasms (Table II). No significant results were observed, and the occasional suggestive findings could not be confirmed. Although the doses administered in the present study were equal to or greater than those employed by the Japanese investigators, our results do not confirm their positive findings (3, 4). This discrepancy could result from the use of different standards for the classification of tumor inhibition; the 16 per cent and 17 per cent inhibition of the rat hepatoma and the Bashford mouse carcinoma observed by Tuboi (4) following the administration of potassium α-naphthaleneacetate would not have been considered as significant in our series. In our experiments the number of animals was restricted because of the large number of compounds tested, and this in turn necessitated the use of more rigid standards concerning tumor growth. It is possible, therefore, that a moderate inhibitory activity might

[1] Traumatic acid: Although this compound will stimulate the proliferation of plant cells under certain specific conditions, it is not usually classed with the other plant hormones.

be demonstrated with some of the compounds that were classed as inactive, because of mild or irregular responses, if they were tested on a larger series of animals and the results then subjected to statistical analyses. Because such observations would probably not be of real significance, experiments designed to test this were omitted for the present.

The difference in results obtained with the two test methods employed in this study may be due to a variety of factors. It appears obvious that the plant hormones never reached a concentration in the animal tissues comparable to that necessary for inhibition *in vitro*. Haagen-Smit, Leech, and Bergren (1) have demonstrated that the ingestion of 3-indoleacetic acid was followed by its excretion in human urine within 2 to 3 hours. Such rapid elimination would not allow a constant supply or a sufficiently high concentration of the hormone to influence greatly the growth of established tumors. The use of some related compounds that would be slowly absorbed in order to insure a prolonged action at relatively high concentrations is indicated from the present study.

TABLE II: EFFECT OF SUBCUTANEOUS INJECTIONS OF VARIOUS PLANT HORMONES ON THE GROWTH OF TRANSPLANTABLE TUMORS

Chemical	Flexner-Jobling rat carcinoma				Mouse fibrosarcoma			
	Amount per injection, mgm.	Number of injections	Total amount injected, mgm.	Results	Amount per injection, mgm.	Number of injections	Total amount injected, mgm.	Results
α-Naphthaleneacetic acid	0.3	16	4.8	0	0.1	16	1.6	0
	1–10	16	110.0	0				
	8–20	15	288.0	0				
3-Indolebutyric acid	0.3	16	4.8	0	0.1	16	1.6	0
	0.4–1.0	16	9.6	0	1.5–3.0	29	54.0	0
	4.5	30	135.0	0				
	20.0	16	320.0	0				
Sodium ω-1-naphthoxyvalerate	1.0–1.5	34	46.0	0	0.5	34	17.0	0
Sodium ω-2-naphthoxyvalerate	1.0–1.5	34	46.5	0	0.25–0.5	34	13.0	0
Sodium ω-2-naphthoxyheptanoate	1.0–1.5	32	39.5	0				
Sodium S-(1-naphthyl)thioglycolate	3.0	16	48.0	0	1.0	16	16.0	0
3-Indoleacetic acid	0.3	16	4.8	0	0.1	16	1.6	0
	1–10	16	110.0	0				
	12–20	11	212.0	0				
Sodium 2-naphthoxyacetate	3.0–4.5	34	138.0	0	0.75–1.5	34	39.0	0
Sodium 2-(4-chlorobenzoyl)benzoate	3.0	16	48.0	0	1.0	16	16.0	0
Sodium β-1-naphthoylpropionate	7.5	16	120.0	0	2.5	16	40.0	0
	15.0	16	240.0	0	5.0	16	80.0	0
Sodium 1-naphthoxyacetate	3.0	16	48.0	0	1.0–3.0	16	26.0	0
	6.0	10	60.0	0	2.0	16	32.0	0
	5.0–15.0	16	140.0	0	5.0	16	80.0	0
Sodium β-1-naphthoxypropionate	2.0–3.0	34	92.0	0	1.0	34	34.0	0
Disodium 2-dodecene-1,10-dicarboxylate	6.0	16	96.0	0	2.0	16	32.0	0
Sodium β-2-naphthoylpropionate	6.0	16	96.0	0	2.0	16	32.0	0
Sodium 1-naphthylmethanesulfonate	6.0	16	96.0	0	2.0	16	32.0	0
Sodium β-naphthylhomophthalimide	6.0	16	96.0	0	2.0	16	32.0	0
Sodium *p*-chlorophenoxyacetate	6.0	16	96.0	0	2.0	16	32.0	0
Disodium 1,4-dihydro-1,4-naphthalene-dicarboxylate	30.0	16	480.0	0	10.0	16	160.0	0
Sodium *o*-chlorophenoxyacetate	30.0	16	480.0	0	10.0	16	160.0	0
Sodium γ-1-naphthylbutyrate	6.0	16	96.0	0	2.0	16	32.0	0
N-(*p*-chlorophenyl)glycine hydrochloride	0.3	16	4.8	0	0.1	16	1.6	0
β-1-Naphthoxyethanol	0.1	32	3.2	0	0.01–0.05	28	0.45	0
β-1-Naphthylethanol	0.9	35	31.5	0	0.15–0.3	34	6.3	0

SUMMARY

Twenty-three plant hormones were tested for their effect upon the takes and subsequent growth of the Flexner-Jobling rat carcinoma, the Walker rat carcinosarcoma 265, and on a transplantable fibrosarcoma of the mouse. The following results were obtained:

1. When tumor mince was allowed to stand before inoculation in direct contact with the plant hormones a pronounced decrease in the number of takes and subsequent growth rate was generally observed.

2. Subcutaneous injection of the same substances, however, exerted no significant effect upon neoplasms already established.

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2. Kline, B. E., Wasley, W. L., and Rusch, H. P. Tumor Inhibitor Studies. I. The Effect of Pure Chemical Compounds on Tumor Takes. Cancer Research, **2**:645-648. 1942.

3. Tanaka, A., and Tuboi, S. Über den Einfluss des β-Indolylessigsauren Kaliums auf das Wachstum des Mäusecarcinoms. Gann, **34**:354-355. 1940.

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Abstracts

Experimental Research, Animal Tumors

Quantitative Analysis of Dose-Response Data Obtained with Three Carcinogenic Hydrocarbons in Strain C3H Male Mice. BRYAN, W. R., and SHIMKIN, M. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:503-531. 1943.

The quantitative relations between dose and tumor response were studied for the following carcinogenic hydrocarbons and dosages: 20-methylcholanthrene in serial twofold doses from 0.00024 to 1.0 mgm., and 1,2,5,6-dibenzanthracene and 3,4-benzpyrene both in serial twofold doses ranging from 0.00195 to 8.0 mgm. The hydrocarbons were injected subcutaneously.

Both tumor incidence and latent period were analyzed in their relations to dose, by the use of appropriate biomathematical procedures. The relative carcinogenic potencies and the relative speeds of action of the 3 materials were determined for the conditions of the experiment. Useful data concerning the relations between dose and response are presented in graphic form, and some implications of the various findings are discussed.—F. L. H.

The Carcinogenic Activity of Some New Derivatives of Aromatic Hydrocarbons. I. Compounds Related to Chrysene. DUNLAP, C. E., and WARREN, S. [Harvard Med. Sch., Boston, Mass.] *Cancer Research*, **3**:606-607. 1943.

The carcinogenic activity of five alkyl and alkylene derivatives of chrysene was tested in mice by subcutaneous injection. 5-Methylchrysene showed a high degree of activity, 4,5-methylenechrysene and 5,6-dimethylchrysene were moderately active, and 4-methylchrysene and 4,5-dimethylchrysene weakly or questionably so. Carcinogenic activity was thus demonstrated in several members of a family of hydrocarbons that lacks the 1,2-benzanthracene ring system and bears a structural relationship to estrogenic substances.—Authors' abstract.

Quantitative Experiments on the Production of Subcutaneous Tumors in Strain A Mice with Marginal Doses of 3,4-Benzpyrene. LEITER, J., and SHEAR, M. J. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:455-477. 1943.

Mice were given single subcutaneous injections with marginal doses of 3,4-benzpyrene in various lipid materials. When lard filtrate was the vehicle for 0.1 mgm. of benzpyrene, tumor production varied widely although rapid tumor production in a high proportion of the animals was usually obtained. Lard residue exercised a striking retardation of tumor genesis by benzpyrene. Fractionation of lard filtrate and residue located the retarding influence in the glyceride fractions that were richest in saturated fatty acids of high molecular weight.

Tricaprylin was the most suitable of several other materials that were tested for suitability as vehicles in quantitative experiments. Synthetic preparations of this triglyceride varied in their effect on carcinogenesis; in some instances this appeared to be correlated with the melting points of the specimens. Standard reference experiments were carried out to determine the average rate of subcutaneous tumor production in mice with different dose levels of 3,4-benzpyrene in 7 specimens of tricaprylin as vehicle.

Exploratory experiments were carried out with a large number of substances of biologic origin incorporated in the benzpyrene solution prior to injection. Indications of a retarding effect on tumor production were obtained with some of these substances.

The incidence of induced subcutaneous tumors was about 50% higher in the male mice than in the female.—F. L. H.

Pirólisis del colesterol. Alquitrán cancerígeno del colesterol. [Pyrolysis of Cholesterol. Carcinogenic Tar from Cholesterol.] ROFFO, A. H. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **18**:929-948. 1941.

Oxidation of cholesterol at a temperature of 145-150° C. leads to the formation of a tar-like substance that induces in the rabbit ear a process of carcinogenesis analogous to that induced by tar itself. Ten illustrations are appended.—M. D-R.

Further Observation on Skin Carcinogenesis by a Single Application of 20-Methylcholanthrene. SIMPSON, W. L., and CRAMER, W. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research*, **3**:604-605. 1943.

It is demonstrated for a third strain of mice, the New Buffalo, that a single cutaneous application of methylcholanthrene in benzene can induce malignant tumors. Of 12 effective mice, 2 developed carcinomas and 1 a sarcoma. Of 4 strains tested so far by this method, only 1 has given a negative result. A positive carcinogenic response to a single application of a potent carcinogen is, therefore, not an exceptional phenomenon restricted to one particularly susceptible strain of mice. It is noteworthy that one of the carcinomas had a very long period of induction, 9 months, and that both its morphology and its biological behavior characterized it as a very malignant type of growth.—Authors' abstract.

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Note on the Comparison of Dosage of Methylcholanthrene on the Production of Leukemia and Sclerotic Lesions in Strain Dilute Brown Mice on a Restricted Cystine Diet. WHITE, J., MIDER, G. B., and HESTON, W. E. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:453-454. 1943.

The incidence of leukemia in animals painted 20 times with methylcholanthrene and ingesting either a dog chow or a high cystine diet was essentially the same as that for animals that received 40 to 70 applications of the hydrocarbon. The mean latent period was somewhat longer. The incidence of sclerosis of the aorta was not significantly lowered, while the latent period was definitely lengthened. Decreasing the total dose of methylcholanthrene caused the animals on the low cystine diet to outlive those on the high cystine diet without developing leukemia. The sclerotic lesions of the aorta appear to be associated with the toxic nature rather than the carcinogenicity of the methylcholanthrene.—F. L. H.

On the Reported Production of Tumors by Normal Liver Cells of Mice Bearing Tumors Produced by Methylcholanthrene. DMOCHOWSKI, L. [Imperial Cancer Research Fund, Mill Hill, England] *Cancer Research*, **3**:608-609. 1943.

An attempt was made to duplicate the results of Selle, Brindley, and Spies.

Mice of the Strong A, C3H, RIII, and Bagg high cancer strains; of the S and C57 low cancer strains; and of the C57 × RIII low cancer strain hybrids were injected with liver suspension from mice belonging to RIII, C3H, Strong A, and Bagg strains bearing sarcomas induced by methylcholanthrene.

No tumors were produced in this way during a period of several months.—Author's summary.

Cancerogenic Extracts from Human Liver. STEINER P. E. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:267-268. 1942.

Tests in mice, not complete, indicate that among 31 livers from cancer-bearing persons the nonsaponifiable lipid fraction extracted from 5 showed carcinogenic activity. Of similar extracts of 13 noncancerous livers, 1 induced sarcomas in mice. Attempts are being made to isolate and identify the carcinogen.—M. E. H.

Initiation of Secretory Changes in Transplanted Mammary Adenocarcinoma of the Rat by Pituitary Lactogenic Hormone. EISEN, M. J. [Coll. of Physicians and Surgeons, New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **51**:260-262. 1942.

Daily injection of 40 to 80 crop gland units of pituitary lactogenic hormone for 4 to 6 days into tumor-bearing rats nursing their young induced secretory changes in the transplanted mammary adenocarcinoma. The hormone was without effect on tumors borne by nonnursing females or normal males. Progesterone also had no influence; nor did it affect, when given simultaneously with estradiol dipropionate, the secretion induced in the malignant cells by the latter substance alone. Pituitary lactogenic hormone failed to induce secretion in the tumor cells of animals previously treated with progesterone, and did not augment the changes produced by the combined administration of progesterone and estradiol.—M. B.

The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **3**:569-577. 1943.

About 80% of turkeys within the first 10 weeks after hatching were found to respond to intravenous injections of Rous sarcoma virus and the same was true for guinea fowls within the first 5 weeks of life. When cell suspensions were injected the incidence of tumors rose to 100 and 88% respectively. A few adult pheasants, injected with cells, also proved to be highly susceptible, while newly hatched or older pigeons were found wholly refractory to both virus and cells. The tumors induced were maintained through 4 passages in turkeys and 6 in guinea fowls by inoculation of tumor extracts or cell suspensions, but the growths were less vigorous, especially when older animals were injected. Both original and transplanted tumors in guinea fowls and in most turkeys were but slightly viscid, very collagenous, and had a pattern approaching that shown by the duck variants of the Rous virus. The tissue affinities of the viruses also were characteristic. Frequently in turkeys and occasionally in guinea fowls, periosteal and endosteal tumors developed, and in the latter hosts the spleen and liver were always typically and almost exclusively involved. The species affinities of the virus were not modified by the adaptation of the tumors to the new species as was the case in ducks. The viruses of the turkey and guinea fowl tumors regularly infected adult chickens, but they showed in these hosts the same tissue affinities, periosteum and endosteum, and induced collagenous tumors much as in their respective homologous hosts. The disease in chickens showed other features in addition whereby it could be differentiated from that induced in them by the original Rous virus. It is concluded that the virus of the Rous sarcoma of chickens has undergone variations in turkeys and guinea fowls and the characteristics of these variants are discussed, especially in relations to the duck variants previously obtained.—Author's abstract.

Growth of a Chicken Sarcoma Virus in the Chick Embryo in the Absence of Neoplasia. MILFORD, J. J., and DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **3**:578-584. 1943.

The virus of the Rous sarcoma of chickens injected intracelomically or intravenously into 3, 6, and 13 day old chick embryos multiplied in these hosts without eliciting tumors, but produced hemorrhagic lesions that were serially transmitted to other embryos in 6 successive passages, again without induction of neoplasia. The injection of extracts of these embryos, however mild, into chicks and pullets resulted in tumors of the ordinary type, but embryos in which no lesions developed apparently contained no transmissible virus. Tumors in the chorioallantois developed in only 2 of the 64 embryos that developed lesions. Thus such growths may coexist with typical hemorrhagic lesions in the embryo proper. Microscopic study of the hemorrhagic lesions disclosed the presence of destructive changes in the vessel wall and adjacent connective tissue and confirmed the absence of neoplasia. It is concluded that viruses inducing sarcomas in chickens can behave as ordinary cell-destroying viruses in very susceptible hosts.—Authors' abstract.

Transformation of Virus of Rabbit Fibroma (Shope) into That of Infectious Myxomatosis (Sanarelli). Houlihan, R. B. [Univ. of Virginia Sch. of Med., Charlottesville, Va.] *Proc. Soc. Exper. Biol. & Med.*, **51**:259-260. 1942.

A 10% suspension of a dermal tumor of virulent myxoma virus was heated for 35 minutes at 60° C. in a water bath and kept in sealed ampoules at 4° C. until used. This tissue suspension was used for the following:

1. Serial testicular passage in rabbits (New Zealands, weighing about 4½ pounds each).

2. Mixture with (a) active fibroma virus, (b) attenuated fibroma virus, (c) diverse agents producing reduction, inflammation, increase in virulence; and subsequent testicular injection or serial passage of these mixtures.

It was found that transformation of fibroma virus into myxoma virus was obtained only when heat-inactivated myxoma virus plus active fibroma virus was passed serially through rabbits.

Further data are required to show whether fibroma virus reactivates heat-inactivated myxoma virus or is actually transformed into myxoma virus.—M. B.

Influence of Hybridization upon the Occurrence of Mammary Tumors in Mice. Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:359-365. 1943.

The general problem is the determination of the characteristics of various inbred strains of mice and their hybrids so that the suitability of each for certain problems in experimental cancer will be known. The present experiments indicate that hybrids derived from crossing together the low mammary tumor strains C57 black and I are more susceptible to the mammary tumor inciter in strain C3H milk than is either parental strain.—G. W. W.

The Effect of Temperature upon Ultraviolet Carcinogenesis with Wave Lengths 2,800–3,400 A. Bain, J. A., Rusch, H. P., and Kline, B. E. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Cancer Research*, **3**:610-612. 1943.

The effect of temperature upon ultraviolet carcinogenesis in mice, with wave lengths 2,800–3,400 Å was investigated. The wave lengths were isolated by means of a special filter and the mice were placed, during irradiation, in chambers held at the desired temperature. The rate of tumor development was significantly greater at 35–38° C. than at room temperature. There was little difference, however, in the rate of carcinogenesis at 3–5° C. and at room temperature.—Authors' abstract.

Wavelength Dependence of Tumor Induction by Ultraviolet Radiation. Blum, H. F. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:533-537. 1943.

The effectiveness of ultraviolet radiation of different wave lengths in inducing tumors of mouse skin was measured, the wave lengths being varied by means of cut-off filters. The maximum effectiveness lies between 2,600 and 3,000 Å. Since the maximum absorption of nucleic acids is at about 2,650 Å, and that of proteins is at about 2,800 Å, it is inferred that one of these is the substance primarily affected by radiation. In all experiments of this type the screening action of the corneum must be taken into account.—H. Q. W.

Effect of Intensity on Tumor Induction by Ultraviolet Radiation. Blum, H. F. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:539-543. 1943.

Male albino mice of strain A were exposed to ultraviolet radiation 5 days per week. The animals were divided into groups receiving the same total dose at widely different intensities. Tumor induction was found to be independent of intensity above a certain critical intensity, but below the critical value the carcinogenic effectiveness decreased sharply. It is suggested that the intensity of the ultraviolet component of sunlight in most parts of the world lies below the critical tumor-inducing value for human skin, and that human skin tumors due to exposure to the sun are uncommon for this reason.—H. Q. W.

Some Fundamental Aspects of Tumor Development Illustrated by Studies with Ultraviolet Radiation. Blum, H. F. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:569-581. 1943.

Male strain A mice were exposed to ultraviolet irradiation, and the time required for the development of tumors up to a preselected standard size was determined. Some animals were exposed at regular intervals until tumors appeared. In other groups of animals the exposures were discontinued after varying times, or were interrupted for about 30 days after 1 or 2 months and then resumed. The results indicated that the changes leading to tumor formation were initiated after a very few exposures, but that subsequent exposures accelerated the process. Rest periods resulted in a slowing, but not in a complete cessation, of the development of the changes leading to the appearance of the tumors. There is an elaborate mathematical analysis of the data.—H. Q. W.

Relation between Radiation Effects and Cell Viability as Indicated by Induced Resistance to Transplanted Tumors. Goldfeder, A. [New York Univ. Med. Coll., New York, N. Y.] *Radiology*, **39**:426-431. 1942.

In previous work the author showed that fragments of mouse tumor exposed to 2,200 to 5,000 r of roentgen rays usually failed to grow when implanted in mice but that these hosts became refractory to subsequent implants of nonirradiated tumor. A dose of 60,000 r was necessary to inhibit the growth of tumor cells in tissue culture. The present paper extends these results.

Fragments of mouse sarcoma 180 were irradiated *in vitro* with various doses of roentgen rays and then tested for their ability: (1) to produce tumor on implantation and (2) to induce "immunity" to a second implantation of nonirradiated tumor cells. Tumor fragments treated with doses of 800 to 2,000 r produced tumors on implantation in the majority of mice but 95 to 100% of the survivors were refractory to further transplants. Implants exposed to 2,200 to 5,000 r failed to produce tumors with a few exceptions but conferred tumor resistance on only 80 to 85% of the survivors. The implantation of tumor fragments previously exposed to 60,000 r produced no tumors and did not alter susceptibility to subsequent tumor implantation.

It is concluded that the factor in mouse sarcoma 180 which produces resistance to reinoculation is destroyed by doses of 60,000 r. Doses between 4,000 and 5,000 r

prevent tumor proliferation *in vivo* but do not destroy this factor. The author is not prepared to propose the injection of tumor tissue, properly attenuated by radiation, as a treatment for human cancer but suggests that the delayed appearance of metastases in some cases after irradiation of a primary tumor may represent an acquired resistant state.—C. E. D.

Peculiar Growth Lesions in Frogs Induced by Irradiation of Sperm Cells with X-Rays. HENSHAW, P. S. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:409-417. 1943.

Frog embryos (*Rana pipiens*) that developed from normal untreated eggs fertilized with irradiated sperm displayed numerous abnormalities. The most important of these were: (1) an almost complete lack of differentiation in some embryos, and (2) a tendency of the ectoderm to form papillomatous growths. These growths showed anaplasia but not invasiveness. There was no evidence that they were malignant, but the possibility of malignancy could not be excluded.

The growth lesion and other abnormalities resulted apparently from changes occurring in the nucleus of the irradiated sperm, for they appeared in the embryos only so long as the male nucleus remained functional. Doses of 50,000 r or more affected the sperm nucleus so severely that it took no part in development. However, the sperm cells remained motile and activated the eggs, which then developed gynogenetically without displaying the growth abnormalities.

Dosages given to sperm in these experiments ranged from 15 to 500,000 r. The smallest dose (15 r) produced abnormalities in 5% of the embryos. With increasing doses the proportion of embryos developing abnormally and the severity of the abnormalities increased. This trend was reversed at 50,000 r in eggs that developed gynogenetically after activation with sperm cells whose nuclei took no part in further development. Doses up to 200,000 or 300,000 r had no effect on the cleavage of eggs activated with the treated sperm; 300,000 to 400,000 r were required to render the sperm immotile.—R. W. B.

Neoplasms and Other Lesions of the Eye Induced by Ultraviolet Radiation in Strain A Mice. LIPPINCOTT, S. W., and BLUM, H. F. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:545-554. 1943.

Neoplasms and other lesions of the eye induced by ultraviolet radiation are described, attention being focused on the changes that may accompany or precede the development of neoplasms. Much of the material for study has been obtained during investigations on the induction of cutaneous tumors. Pathologic changes are superficial, being confined primarily to the cornea. Hyperplasia of the epithelial and connective tissue elements, and vascularization are observed. There may be inflammatory changes, and iris and lens may be involved secondarily. The tumors observed have been sarcomas and hemangioendotheliomas of the substantia propria. Changes in the epithelium of the cornea suggest that carcinomas may occur at times. The possible etiologic role of sunlight in producing lesions of the human eye is discussed.—C. J. L.

Nueva técnica de la determinación de la fluorescencia en el Instituto de medicina experimental. [New Technic for Determining Fluorescence in the Institute of Experimental Medicine.] ROFFO, A. E., JR. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer,* **18**:975-990. 1941.

A detailed description of the method used. Nine illustrations are appended.—M. D-R.

Studies of Irradiation Effects on Cancer Cells. I. Preliminary Report. The Production of Liver Cancer in White Rats. WILLIAMS, G. Z., CARY, M. K., and WILLIAMS, J. T. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly,* **69**:93-95. 1942.

An average incidence of 80% of cancers of the liver was obtained in 2 strains of white rats by the feeding of dimethylaminoazobenzene. No significant reduction of incidence or mortality from cancer of the liver was produced by the administration of 2,000 r of 200 kv. x-ray.—M. E. H.

Degradation of Cystine by Normal Liver but Not by Transplanted Hepatomas. GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:491-494. 1943.

Cystine, cysteine, peptides of cystine, and mixtures of cystine with other amino acids were incubated with extracts of normal liver and of transplanted rat and mouse hepatomas. Cystine and related compounds were rapidly degraded by the normal livers of rats and of mice but were not at all attacked by extracts of the hepatomas.

The enzyme complex in the liver responsible for the degradation of cystine and its possible role in tumor induction under certain dietary conditions are discussed.—F. L. H.

Hydrogen-Ion Concentration of Normal Liver and Hepatic Tumors. KAHLER, H., and ROBERTSON, W. v. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:495-501. 1943.

Because many comparisons have been made of the enzyme activities of normal livers and hepatic tumors, and because the activities of many enzymes are dependent on pH, the authors have measured the pH *in vivo* of rat hepatoma 31, spontaneous rat hepatoma, mouse hepatomas of several types, and the livers of normal and hepatoma-bearing rats. The technic of the pH measurements is described. The average pH of the tumors in 10 fasted rats was 6.99; that of the livers of 12 normal rats was 7.39; and that of the livers of 9 hepatoma-bearing rats was 7.30. Intraperitoneal injection of glucose caused the pH of the rat hepatomas to fall to an average pH of 6.43 in 3 hours, but did not affect the pH of liver. Glucose injection caused an irregular increase in the lactic acid content of the tumors, but not of the livers. Results with mouse hepatomas were variable, but the average pH of the tumors was 6.74 before, and 6.22 after, the injection of glucose.—H. Q. W.

Growth Rate and Number of Spontaneous Mammary Carcinomas and Riboflavin Concentration of Liver, Muscle, and Tumor of C3H Mice as Influenced by Dietary Riboflavin. MORRIS, H. P., and ROBERTSON, W. v. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:479-489. 1943.

Riboflavin was found essential for the growth of normal young mice and for the maintenance of body weight of adult normal and tumor-bearing mice. The food intake of the animals was not greatly influenced by a deficiency of riboflavin.

The growth rate of spontaneous mammary carcinomas was decreased in the animals on the riboflavin-deficient diet. The most extensive effect on the reduction in tumor growth occurred in animals after 3 weeks on the deficient diet and roughly coincided with the riboflavin depletion of the tumor. No appreciable difference in tumor growth rate was observed between mice on a maintenance level of riboflavin and those on a riboflavin-supplemented diet. Feeding a high level of riboflavin definitely increased the number of spontaneous tumors in the tumor-bearing animals.

There was no correlation between the total size of the tumor and the length of survival of the animal on any of the different levels of riboflavin. A significant decrease was observed in survival of tumor-bearing, riboflavin-deficient mice as compared with mice receiving riboflavin. The survival period was increased by placing the tumor-bearing animals on a riboflavin-deficient diet for 3 weeks and then supplementing the diet with riboflavin.

The riboflavin content of the liver of normal adult and of tumor-bearing mice decreased about 50% on the deficient diet in 4 weeks without much further decrease after 6 weeks. The riboflavin content of muscle decreased about a third during the same period, while that of the tumor decreased nearly 50%, but more slowly than in liver and muscle.—F. L. H.

Tissue Metabolism Studies on Bone Marrow. Consideration in Relation to Tumor Metabolism. WARREN, C. O. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research*, **3**:621-625. 1943.

Bone marrow respiration and glycolysis are reviewed and new data added in order to consider them with reference to the 8 criteria of a tumor type of metabolism listed by Burk. Myeloid, but not erythroid cells, fulfill 7 of the 8 criteria; only the relatively high R. Q. (about 0.96) serves to distinguish these cells metabolically from malignant cells. The succinate and *p*-phenylenediamine tests of Craig, Bassett, and Salter appear not to be of value in this connection, and their general applicability is questioned. Also, more evidence is presented to support the view that growth and glycolysis are not necessarily related. The implications of these studies with reference to the metabolism of leukemic cells is discussed.—Author's abstract.

Effect of a Diet Relatively Low in Cystine on the Production of Spontaneous Mammary-Gland Tumors in Strain C3H Female Mice. WHITE, J., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:449-451. 1943.

Litter mate virgin C3H mice at the time of weaning were divided into 2 groups. One was placed on a relatively low cystine diet, and these mice failed to develop spontaneous mammary gland tumors, even at the end of 22 months. The second group was put on a high cystine diet and the animals developed tumors to the same extent and in about the same average time as did mice of the same strain on a dog chow diet. Mice on the low cystine diet showed irregular, and in many cases complete absence of, estrous cycles. This may be associated with the failure to develop mammary gland tumors.—F. L. H.

Action of Bacterial Toxins on Tumors. III. Some Biological Properties of Purified Salmonella typhimurium Endotoxin. ZAHL, P. A., and HUTNER, S. H. [Haskins Lab., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **52**:116-118. 1943.

Salmonella typhimurium endotoxin was extracted with acetone, phenol, and formamide. When tested on mice, the various fractions were found active in respect to: (1) lethality, (2) the induction of hemorrhage in transplanted tumors (mouse sarcoma 180), (3) the production of antibodies that protected mice against the toxic material.—M. B.

Growth and Regression of Frog Kidney Carcinoma Transplanted into the Tails of Permanent and Normal Tadpoles. BRIGGS, R., and GRANT, R. [Lankenau Hosp. Research Inst., Philadelphia, Pa., and McGill Univ., Montreal, Canada] *Cancer Research*, **3**:613-620. 1943.

Kidney carcinoma from adult frogs (*Rana pipiens*) transplanted into the dorsal mesenchyme of the anterior third of the tails of normal young tadpoles, grows well and maintains its characteristic structure during early larval development, but regresses rapidly as the hosts approach metamorphosis. Carcinoma implants in the tails of nonmetamorphosing (thyroidectomized or hypophysectomized) tadpoles follow the same growth-regression sequence. The proportion of the effective total of implants that take and grow (53 to 60%), the proportion that regress after growth (95 to 100%), the rate of implant growth, the maximum size attained, and the rate of regression, are not significantly different in nonmetamorphosing tadpoles compared with normal control tadpoles.

The changes in the host tail that bring about tumor regression during later larval development are not known. Regression is associated with an accumulation of spindle-shaped mesenchyme cells, and occasionally of numerous small round cells, around the implant. This type of cell response may be a causative factor. The present work indicates that this or other unknown factors are effective against cancerous as well as incompatible normal tissues, and are developed in the absence of thyroid or pituitary, and of the metamorphic alterations with which these glands are associated.—Authors' abstract.

Changes Induced in a Strain of Fibroblasts from a Strain C3H Mouse by the Action of 20-Methylcholanthrene. (Preliminary Report.) EARLE, W. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:555-558. 1943.

Five tissue culture strains of fibroblasts derived originally from the subcutaneous and adipose tissue of a 100 day old mouse (C3H) were subjected to the influence of methylcholanthrene in a concentration of 1 γ per cc. of culture media for periods varying from 6 to 406 days. A retarda-

tion in rate of cell proliferation was observed after a very few days of treatment with the carcinogen. At the end of about 40 days' exposure, changes appeared in the individual cells and also in the culture morphology. Terminal processes decreased in length, and the cells became increasingly coherent until the cultures took on somewhat the appearance of epithelial sheets. These changes were maintained after the carcinogen had been withdrawn.

After varying periods of time had elapsed, up to as much as 1 year following discontinuance of the carcinogen treatment, cells from the 5 treated strains were injected into young adult C3H mice. Tumors arose at the sites of inoculation within periods as short as 9 days. The percentage of positive takes varied from 72% for one strain of cells originally treated 6 days with the carcinogen to only 7.6% for two other strains treated with the carcinogen for 184 and 406 days respectively.

Control cultures remained unchanged for long periods, but ultimately these too showed cellular and cultural alterations similar in character, though not in extent, to those shown by the cultures subjected to methylcholanthrene. Injected into C3H mice, these altered control cells yielded from 7.7% for one strain to as high as 86% positive takes for other strains. The question whether some trace contamination of carcinogen might be responsible for this change in and behavior of the control cultures will be discussed in a later report. Such contamination is considered likely.

The author indicates an apparent relation between the degree of cellular abnormality in the cultures and the propensity of the cells to develop into tumors upon inoculation into animals. Cells from the most abnormal, and least abnormal, cultures grew in only a low percentage of instances.—K. P.

Morphology of Sarcomas Derived from Fibroblasts Previously Treated with 20-Methylcholanthrene in Vitro. (Preliminary Report.) Nettleship, A. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:559-561. 1943.

Tumors that developed in C3H mice at the site of inoculation of methylcholanthrene-treated fibroblasts or altered control cells appeared in the gross as hard, globular masses firmly attached to the muscles and skeleton. Central necrosis was slight in small tumors, more pronounced in larger specimens. There was extensive involvement of pelvic and abdominal organs by direct extension, possibly also by metastases. Frank metastases were found in lungs and mediastinum.

Microscopic study revealed the tumors to be typical mouse spindle cell sarcomas of both the large and small cell varieties. The metastases displayed the characteristics of the primary tumor. Metastasis appeared to take place through the blood vessels.

Although all these sarcomas arose from tissue cultures derived from connective tissue, their morphology suggests many varieties of sarcoma, such as neurogenic, angiomatous, myxomatous, and round and giant cell types.

The malignant properties of the 5 cell strains, including methylcholanthrene-treated and controls, were analyzed in terms of irregularity of cell pattern, invasiveness, mitoses, giant cells, and metastases. This analysis leads the author to conclude that cells subjected to short exposures of carcinogen and showing only slightly distorted cell structure (as in cultures exposed for 6 days, and also in controls) possess a low degree of malignancy. More prolonged treatment yields cells showing similar slight abnormalities but maximum malignancy. Exposures beyond this produce greater cell changes but less active invasive and metastasizing properties.—K. P.

Studies in Vitro on the Physiology of Normal and of Cancerous Cells. I. The Effect of High Temperature and of Moccasin Venom on the Viability of Rabbit Lymphocytes and Polymorphonuclear Leukocytes as Determined by the Method of Unstained Cell Counts. Schrek, R. [Veterans' Administration, Hines, Ill.] *Arch. Path.*, **35**:857-868. 1943.

Making use of the imperviousness of cells to eosin as an indicator of viability, the author found that the polymorphonuclear leukocytes of the rabbit survive a longer period than do the lymphocytes at temperatures of 56°, 50°, and 45° C., and also that moccasin venom has the ability to kill, agglutinate, and lyse lymphocytes but has little or no effect on the viability and motility of the polymorphonuclear leukocytes.—J. G. K.

Recent Trends in Cancer Research. Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *Minnesota Med.*, **25**:697-703. 1942.

Four directions in which cancer research has developed during the past few years are discussed under the following headings: (1) Inbred animals and their use in cancer studies. (2) The study of naturally occurring products in the body that are of importance in the occurrence of mammary cancer in mice. (3) Chemical substances that produce cancer in experimental animals. (4) Recent work on virus-induced tumors.—J. L. M.

The Practical Significance of Cancer Research. Emge, L. A. [Stanford Univ. Sch. of Med., San Francisco, Calif.] *West. J. Surg.*, **50**:32-36. 1942.

Four main aspects of the cancer problem are discussed in general terms: genetics, extrinsic carcinogenic factors, intrinsic factors, and the destruction or arrest of growth of cancer cells.—M. E. H.

Sex Hormones and Cancer. Twombly, G. H. [New York, N. Y.] *Connecticut M. J.*, **6**:822-827. 1942.

The author discusses under the heading of "sex hormones" the gonadotrophic hormones of the pituitary, the estrogenic hormones of the ovaries, those of the adrenal and testis, and the gonadotrophic hormones of the placenta. The hormones of the ovary and the testis will produce cancers in experimental animals when given over long periods of time; so far it has not been proved that this may occur in man. Cancer of the breast has been shown to regress following removal of the ovaries, and cancer of the prostate following surgical castration.—M. E. H.

Carcinoma espontáneo de un aguti. [Spontaneous Carcinoma in an Agouti.] Carini, A. [Laboratorio Paulista de Biología de São Paulo, Brazil] *Bol. Inst. de biol. de São Paulo*, **58**:1183-1185. 1941.

A squamous cell carcinoma in the skin of the back of an agouti is reported. Transmission was not attempted.—M. D-R.

Clinical and Pathological Reports

Etiology

Composite Factors in the Genesis of Cancer. MacCarty, W. C., Sr. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:337-340. 1941.

The author presents his concept of the genesis of cancer, which is based on a personal study of various phases of the disease over a long period of years. Many specific agents or factors may cause destruction of tissues. The author believes this destruction to be the first essential biological causative factor. Alone it does not always produce cancer, but it is frequently followed by lipoidal or fatty degeneration, some of the products of which may alter cellular surface conditions that bring about cellular hypertrophy or hyperplasia. When this occurs the lipoidal products must be removed by certain mechanisms of the body or they continue such stimulation. Lipolysis occurs normally in the body and is usually complete. But sometimes it is incomplete, and the latter condition may be a possible cause for the physical stimulation of cellular regeneration with or without complete differentiation or, as one sees in the initial stages of cancer, little or no differentiation. The migration of cells, which constitutes the chief characteristic of cancer, is believed to be the expression of a fundamental biological defensive phenomenon; it is looked upon as the result of an interference with the food supply of the hyperplastic cells.—J. L. M.

Cancer—A Deficiency Disease? Macdonald, H. [Evanston, Ill.] *Illinois M. J.*, **82**:210-214. 1942.

The author offers the suggestion that cancer may be due to the absence from the diet of some constituent of certain visceral organs, notably of brain and stomach tissue.—M. E. H.

Heredity

Symposium on Gynecologic Cancer. III. Heredity as an Etiological Factor in Cancer. Macklin, M. T. [Univ. of Western Ontario Med. Sch., London, Canada] *West. J. Surg.*, **50**:439-444. 1942.

According to the author, heredity is not an aid in preventing cancer, as the disease cannot be bred out. The majority of patients develop cancer after they have ceased producing their families. Heredity may be of importance in that it may become an instrument in the hands of the physician for the achievement of early diagnosis.—M. E. H.

Diagnosis—General

The Diagnostic Value of Hormone Assays. Rakoff, A. E. [Jefferson Med. Coll., Philadelphia, Pa.] *M. Clin. North America*, **26**:1915-1937. 1942.

A comprehensive discussion in which hormonal excretion in patients with tumors is reviewed. Chorionepithelioma of the testis produces large amounts of gonadotropic hormone. Generally these are sufficiently large to give a positive Friedman or Aschheim-Zondek test. After surgical or roentgen therapy, repeated gonadotropin assays are of use in following the course of the disease.

High blood and urine estrogen levels have been found in granulosa cell tumors of the ovaries. In certain tumors and hyperplasias of the adrenal cortex associated with the adrenogenital syndrome increased amounts of estrogens may sometimes occur. There is usually, however, a more striking increase in the androgens and 17-ketosteroids.—J. L. M.

La reacción de Roffo. Su importancia diagnóstica en la clínica del cáncer. Resultados obtenidos en 9005 reacciones. [Roffo's Reaction. Its Importance in the Cancer Clinic. Results Obtained in 9,005 Tests.] Stuckert, B. G. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **18**:1141-1181. 1941.

In tests of 4,818 noncancerous patients, the results could be classified as positive or doubtful in only 9.04% of the cases. Among 4,187 cancerous patients, the results were negative in 18.96%.—M. D-R.

Therapy—General

Report on "Glyoxylide" by the Ontario Commission for Investigation of Cancer Remedies. *Canad. M. A. J.*, **47**:63-65. 1942.

The substance "glyoxylide" is said to have been perfected by, and the formula of it to be the property of, one Dr. William F. Koch, of Detroit, Michigan. It is stated that this substance may benefit or cure not only cancer, but also coronary disease, infections like tuberculosis, anterior poliomyelitis, and leprosy, eczema, hay fever, asthma, endarteritis obliterans, psoriasis, and benign tumors. Its use is urged in all these conditions. It was stated by a nurse in Dr. Koch's employ that 3,000 physicians are administering glyoxylide.

The Commission has not been able to learn how many patients have been treated directly or indirectly by Dr. Koch, nor what percentage have recovered. Inquiry failed to elicit any report made by any recognized authority in the United States that could be of assistance to the Commission. Dr. David H. Arnott, of London, Ontario, communicated with the Commission signifying his willingness to stand as sponsor for the substance. Cases presented as having been treated by Dr. Arnott personally were investigated by the Commission with the following findings: of a group of 10 patients treated by glyoxylide, 2 died of cancer; 2 patients with cancer received, in addition, either surgical treatment or radiotherapy. In the latter it is not possible to decide whether the cure or improvement noted was derived from surgery, radiotherapy, or glyoxylide. In 5 cases, the diagnosis of cancer was either lacking or questionable; 1 case was not of cancer but of endarteritis obliterans and was said to have resulted in recovery.

Through arrangements made by Commissioner Dr. Valin, Dr. Arnott attended in Ottawa and treated 10 patients. In connection with these cases Dr. Valin and the medical observer in charge reported as follows: "out of nine patients with positive biopsies for cancer and treated with glyoxylide nine, or 100 per cent, are dead. The other case who is well did not have cancer."

Three other sponsors presented cases lacking material value.

Considerable weight was attached by Dr. Arnott to a communication by Professor Maisin, of the University of Louvain, Belgium. In a letter, Dr. Maisin stated that over a period of 5 years he had seen cancer disappear in animals and in men, as a result of the use of glyoxylide.

As regards laboratory experimentation, Dr. Arnott has from time to time voiced his desire to cooperate but the Commission has never been able to obtain a sample of the substance in question or to observe or learn its exact method of preparation. Arrangements made for laboratory tests were never carried out, owing to objections by Dr. Arnott.

A careful review of all the evidence presented at this date fails utterly, in the opinion of the Commission, to support the claim made on behalf of the Koch treatment that it is either a remedy or a cure for cancer.—A. C.

Radiation—Diagnosis and Therapy

The Indications for Roentgen Therapy. Benjamin, E. W. [Providence, R. I.] *Rhode Island M. J.*, **25**:182-184. 1942.

A brief survey of the general indications for roentgen irradiation in neoplastic disorders. The importance of cooperation between radiologist, clinician, surgeon, and pathologist is stressed.—M. E. H.

The Indications for Radium Therapy. Fox, G. R. [Memorial Hosp., Pawtucket, R. I.] *Rhode Island M. J.*, **25**: 184-187. 1942.

A short and general discussion on the fields of usefulness of radium in the treatment of cancer.—M. E. H.

Surgical Treatment of Roentgen and Radium Dermatitis. Ghormley, R. K. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:69. 1941.

A review of 97 cases involving patients who came to the Mayo Clinic for treatment of roentgen and radium dermatitis revealed the fact that in 20 cases, or 20.6% of the total number, epitheliomas had developed in regions affected by actinodermatitis. This supports the well known observation that roentgen dermatitis is a potentially malignant lesion. Although malignant lesions developed in only a portion of the instances of this type of dermatitis, the incidence is high enough to make adequate treatment imperative in all cases, particularly those in which ulceration is present. The treatment of choice, according to the author, is wide excision followed by a skin graft, preferably of the split type. Excision and primary closure of small areas of roentgen dermatitis with or without ulceration are possible when sufficient mobility of the surrounding skin is present to permit its being brought together.—J. L. M.

The Cyclotron and Its Medical Implication. Editorial. Hempelmann, L. H., Jr. *Radiology*, **39**:627-628. 1942.

Two products of the cyclotron of interest to medical men are the artificial radioactive elements and neutron rays. Radioactive phosphorus is being used experimentally in the treatment of malignant disease since it accumulates in tumors, and thus the malignant cells are exposed to larger doses of damaging radiation than are the surrounding normal tissues. It is too early to claim major advantages from this form of therapy; its chief importance lies in its unexplored possibilities.

The possibilities of neutron therapy are even less well understood, but the preliminary clinical and experimental work indicates that the effect of neutrons on tissues is different from that of other forms of radiation. Since neutrons have a greater effect than x-rays on cells in the resting phase, they may prove more effective in the treatment of slowly growing tumors.—C. E. D.

Irradiation Treatment of Carcinoma of the Cervix. Kerr, H. D. [State Univ. of Iowa Coll. of Med., Iowa City, Iowa] *Wisconsin M. J.*, **41**:34-40. 1942.

The author reviews the prognosis, results, and reactions associated with combined radium and roentgen ray therapy of carcinoma of the cervix.—M. E. H.

The Roentgen Diagnosis of Lesions Involving the Ileum, Cecum and Proximal Ascending Colon. Pendergrass, E. P., and Chamberlin, G. W. [Philadelphia and Reading, Pa.] *Am. J. Roentgenol.*, **48**:16-26. 1942.

The difficulties involved in diagnosing lesions of the ileum, cecum, and ascending colon are illustrated by 13 brief case histories and 9 roentgenograms. Among the possibilities that must be considered are benign and malignant tumors, nonspecific granulomas, inflammatory processes, and congenital or acquired adhesions.

Benign tumors are more common in the small intestine, produce a rounded defect, and, if pedunculated, may cause intussusception. Multiple tumors of the ileum suggest carcinoid. Carcinomas of the cecum or ascending colon are the most common malignant tumors of the lower right quadrant. The medullary type may produce polypoid intraluminal masses, while smooth annular constrictions are more common in the scirrhous form. Cancer of the terminal ileum is rare but must be differentiated from inflammatory lesions. Stenosis, obstruction, and fixation are common. Intestinal tuberculosis is usually associated with pulmonary disease and is more frequent in children.

Increased intestinal irritability is a valuable sign. Multiple ulcerations, hyperplastic mucosal defects with some fixation and narrowing may be seen.

Regional ileitis is usually associated with decreased motility, early disappearance of mucosal pattern, and later development of a rigid narrow lumen. An appendiceal abscess may simulate the smooth outline of an extrinsic tumor. Accurate clinical data together with knowledge of the anatomical characteristics of the possible lesions are essential to correct roentgen diagnosis in this difficult region of the intestine. The barium meal, the opaque or double contrast enema, and study with the Miller-Abbott tube all have advantages and disadvantages.—C. E. D.

A Study of Radiological Treatment of Cancer of the Cervix. Reinhard, M. C., Goltz, H. L., and Schreiner, B. F. [State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] *Radiology*, **39**:144-150. 1942.

A study is presented of 557 patients with carcinoma of the cervix, including all those with clinical grades I, II, and III, admitted to the State Institute for the Study of Malignant Diseases from 1931 to 1935. A graph of the

age distribution is given and shows a peak at 48 years. Another graph shows the percentage survival at 6 month intervals for 5 years. Four hundred and fifty-seven cases, treated chiefly with radium, were suitable for further statistical analysis. The survival time was less in those patients who developed tumors at an early age and in those who required supplemental irradiation subsequent to the initial treatments. The sum of the doses of radium, roentgen rays, and radon was computed, for each patient, in terms of roentgens delivered to a theoretical point 2 cm. from the midline of the pelvis. Within the range of 4,000 to 8,000 r there was no evidence of better results with the higher doses. The 5 year survival rate was 75% in clinical groups I and II combined, and 55% in group III. Isodose and dosage distribution curves are presented to show that the technic used failed to deliver adequate radiation to the lateral portions of the pelvis.—C. E. D.

Sobre la fuente de ultravioletas utilizada en el Instituto de medicina experimental. [The Source of Ultraviolet Radiation Employed in the Institute of Experimental Medicine.] Roffo, A. E., Jr. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer,* **18**:962-973. 1941.

Detailed description of the apparatus used. Five illustrations are appended.—M. D-R.

The Roentgenological Appearance of Extramucosal Tumors of the Esophagus. Analysis of Intramural Extramucosal Lesions of the Gastrointestinal Tract in General. Schatzki, R., and Hawes, L. E. [Massachusetts Gen. Hosp., Boston, Mass.] *Am. J. Roentgenol.,* **48**: 1-15. 1942.

Six cases of intramural lesions of the esophagus are presented, and the aspect of roentgenologic diagnoses is discussed. There were 2 cysts, 1 neurofibroma, and 3 lesions not diagnosed histologically. The features common to these lesions were the presence of a mass sharply outlined in the relief roentgenograms and an abrupt, sharp angle where the tumor met the uninvolved wall of the esophagus. The mucosa was preserved but flattened over the bulging mass. These characteristic appearances were often elusive and were seen clearly only when the proper amount of barium was administered and the tumor was viewed exactly face on or in perfect profile.

Experimental roentgenograms are shown of esophagi removed from cadavers and distorted by intramural and extrinsic artificial tumors made of cork, paraffin, or inflated rubber balloons. Foreign masses within the wall of the esophagus or firmly attached to its outer surface produced an identical type of distortion, which was the same as that seen in the patients with intramural lesions. Loosely attached or free extrinsic masses both in living patients and in the experimental preparations produced a filling defect of different character, which lacked clear cut limits. The same principles of differential diagnoses apply to intramural and extrinsic lesions throughout the gatrointestinal tract. Adherent tuberculous lymph nodes or stone in the intraduodenal portion of the common bile duct may simulate intramural lesions. Thirty-seven roentgenograms are reproduced.—C. E. D.

Symposium on Gynecologic Cancer. IV. Radiation in the Treatment of Genital Cancer. Schmitz, H. E. [Chicago, Ill.] *West. J. Surg.,* **50**:445-448. 1942.

Under three headings: (1) carcinoma of the cervix, (2) carcinoma of the uterine body, and (3) malignant diseases of the ovaries, the author outlines the technic of choice for combined radium and roentgen treatment.—M. E. H.

Pyloric Ulcers. Smedal, M. I. [Lahey Clinic, Boston, Mass.] *Radiology,* **39**:200-207. 1942.

Ten cases of pyloric peptic ulcer are presented, and the method of differentiating them from prepyloric and duodenal ulcer is illustrated by reproductions of 24 roentgenograms. The characteristic findings are: deformity of the prepyloric area and base of the duodenal cap, distortion and broadening of the pyloric sphincter, and an ulcer crater usually on the lesser curvature at the sphincter. Localization of these ulcers is important since they are rarely malignant in contrast to prepyloric ulcers with which they may easily be confused.—C. E. D.

The Roentgen Ray Diagnosis of Gliomas. Sterling, H. W. [Veterans' Administration, North Little Rock, Ark.] *M. Bull. Vet. Admin.,* **18**:46-47. 1941-42.

A classification to aid in the identification and localization of gliomas by roentgen ray examination.—M. E. H.

Radium Treatment of Carcinoma of the Cervix. Trapp, E. [Cancer Inst., Vancouver, Canada] *Canad. M. A. J.,* **46**:173-177. 1942.

A critical historical review of the subject, with a description of the treatment adopted at the Cancer Institute of Vancouver, which is based on that developed at the Radiological Institute of Stockholm. Radiation treatment of carcinoma of the cervix represents one of the most successful chapters in the radiation therapy of cancer. The best results are obtained in clinics when all treatments are supervised or carried out by one man.—A. C.

Lymphoblastoma Primary in the Gastrointestinal Tract. Weber, H. M., Kirklin, B. R., and Pugh, D. G. [Mayo Clinic, Rochester, Minn.] *Am. J. Roentgenol.,* **48**:27-37. 1942.

A review of the literature on the roentgen diagnosis of lymphoblastoma of the gastrointestinal tract reveals that few if any dependable criteria have been proposed for differentiating this tumor from other malignant and benign conditions. All cases in the Mayo Clinic files designated as lymphoblastoma, lymphosarcoma, or Hodgkin's disease were reviewed, and 34 were found in which the lesion was primary in the gastrointestinal tract and adequate roentgenological studies were available. The stomach was involved in 25 cases, the small intestine in 3, and the large intestine in 6. In the 25 cases of stomach lesions an unqualified roentgenologic diagnosis of carcinoma was made in 19, in none was lymphoblastoma suggested. Review of the roentgenograms and restudy of several patients after exploratory surgery failed to reveal any neglected clues. The 3 lymphoblastomas of the small intestine were diagnosed respectively as an obstructing lesion, a perforating lesion, and carcinoma. In 1 of the 6 lesions involving the colon a presumptive diagnosis of lymphosarcoma was offered.

In the great majority of cases lymphoblastoma may be diagnosed as neoplastic and usually as malignant, but the authors can offer no suggestions for achieving greater roentgenological accuracy. Gastroscopy may be of some help. Twelve roentgenograms and 3 photographs of gross specimens are reproduced.—C. E. D.

Discussion on the Technique of Radiotherapy. *Proc. Roy. Soc. Med.*, **36**:261-270. 1943.

Discussion by B. W. Windeyer, Constance Wood, Ralston Paterson, and others.—E. L. K.

Discussion on the Value of Irradiation in Association with Surgery in the Treatment of Carcinoma of the Breast. *Proc. Roy. Soc. Med.*, **36**:237-242. 1943.

Discussions by Stanford Cade and others.—E. L. K.

Skin and Subcutaneous Tissues

Superficial Basal-Cell Carcinoma. Airey, F. S. *Proc. Roy. Soc. Med.*, **36**:293. 1943.

Description of a case.—E. L. K.

Cancer Scroti. Chase, P. P. [Providence, R. I.] *Rhode Island M. J.*, **25**:104-106. 1942.

A typical case of chimney sweeps' cancer is reported.—M. E. H.

Cancer of the Face. New, G. B. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:71-72. 1941.

Complete primary removal of a cancer of the face, regardless of the apparent inactivity of the lesion, is essential in order to prevent the pronounced deformities that result from the late removal of recurring disease. When cancer about the face has been treated and the condition has not cleared up entirely, the apparent limits of the disease are indeterminate, and it is only by wide removal with surgical diathermy, particularly if cartilage or bone is involved, that permanent elimination of the growth is obtained.

Early lesions of low grade cancer of the face may be excised and replaced immediately with a free shaved skin graft. This method should be employed only for the inactive lesions in which the possibility of recurrence is slight. After an inactive lesion has been removed, in general it is best to wait before attempting reconstructive surgery until it is fairly well established that the growth will not recur. In the period of waiting an artificial prosthesis is employed to replace the lost part so that the patient may go about his work without appearing unsightly.—J. L. M.

A propósito de un caso de epitelioma nevico con metastasis ganglionar. [A Case of Epithelioma Arising from a Naevus, with Lymph Node Metastases.] Ponce, M. E. [Hospital Rosales, San Salvador] *Arch. Hosp. Rosales*, **34**:737-746. 1942.

A report of a case with a review of the literature.—M. D-R.

Cancer of the Skin. Ronchese, F. [Providence, R. I.] *Rhode Island M. J.*, **25**:61-63. 1942.

Early recognition, prompt and adequate therapy, should make recovery the rule in cancer of the skin.—M. E. H.

Naevocarcinoma (Malignant Melanoma). Wigley, J. E. M. *Proc. Roy. Soc. Med.*, **36**:281. 1943.

Description of a case.—E. L. K.

Nervous System

Intraventricular Meningiomas. Review of the Literature and Report of Two Cases. Abbott, K. H., and Courville, C. B. [Los Angeles County Hosp., Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **7**:12-28. 1942.

The authors have tabulated 50 cases, including their own, of intraventricular tumors reported between 1922 and 1942 that had been identified microscopically. The lateral ventricles proved to be the seat of the tumor in the great majority of cases; only in 4 cases were the tumors primary either in the third or the fourth ventricle. With one possible exception the reported tumors were found to belong to the type of fibrous or fibroblastic meningioma. The fact that only the fibroblastic type is found arising from within the ventricles would seem to disprove the assumption that the fibroblastic meningiomas are of dural origin. The problem of the origin of these tumors is discussed in detail.—A. C.

Medulloblastomas. Concerning the Problems of Spinal Metastasis and Malignancy: A Report of Six Cases and Discussion of the Problems Involved. Abbott, K. H., and Kernohan, J. W. [Mayo Clinic, Rochester, Minn.] *Bull. Los Angeles Neurol. Soc.*, **8**:1-10. 1943.

Medulloblastomas have a tendency to inoculate the cerebrospinal spaces spontaneously and thus form cellular implantations. Only occasionally do these spinal metastatic growths give rise to clinical evidence of their presence. The conditions leading to the dissemination, implantation, and growth of the metastatic elements are discussed.—A. C.

Brain Tumor Masked by Cerebral Arteriosclerosis; Two Cases. Arenson, N., and Ginsberg, S. T. [Veterans' Administration, Roanoke, Va., and Marion, Ind.] *M. Bull. Vet. Admin.*, **18**:322-325. 1941-42.

A report of 2 cases.—M. E. H.

Complete Removal of a Large Intramedullary Tumor (Ependymoma) from the Cervical Portion of the Spinal Cord: Report of Case. Baker, G. S., and Stafford, D. E. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:421-423. 1941.

The case reported is unusual in that the patient not only recovered rapidly from removal of a large intramedullary ependymoma from the cervical portion of the spinal cord, but also showed a remarkable improvement in the use of his extremities, which had been paralyzed before operation.—J. L. M.

Surgical Approach to Meningiomas in the Region of the Sphenoid Ridge Causing Unilateral Exophthalmos. Barens, S. N. [Seattle, Wash.] *West. J. Surg.*, **50**:225-229. 1942.

A further development of the Kroenlein operation is presented.—M. E. H.

Meningiomas of the Spinal Cord. Buchstein, H. F., [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Minnesota Med.*, **24**:539-545. 1941.

Four meningiomas of the spinal cord, removed surgically, are here reported to illustrate the clinical and

surgical aspects of these tumors. Meningiomas constitute one-fourth of all neoplastic spinal cord tumors. They are solitary, benign growths, which arise in the spinal meninges and compress, but do not invade, the spinal cord. Their symptoms are those of spinal cord tumors in general, consisting of varying combinations of nerve root pain and signs of spinal cord compression. A spinal cord tumor may be suspected of being a meningioma when it occurs in the thoracic region of the spine in a woman of adult years and has produced symptoms for 1 to 2 years. Occasionally a positive preoperative diagnosis may be formulated by making a calcified meningioma radiographically visible.

Most meningiomas of the spinal cord may be completely removed surgically with a gratifying restoration of function. The risk attending such operations has been reduced by refinements of technic that necessitate accurate preoperative localization of the tumor. The subarachnoid injection of lipiodol is the most frequently employed localizing method and is the only effective method in many cases. Other and simpler methods are also available, as is demonstrated by the present series of cases in which 1 tumor was made visible by direct radiography, 1 was found by the method of multiple spinal punctures, and the remaining 2 were demonstrated by air myelography.—J. L. M.

The Parasagittal Syndrome. Experiences with Degenerative Lesions of the Brain Simulating the Effects of Parasagittal Meningioma. COURVILLE, C. B. [Coll. of Med. Evangelists, Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **8**:31-34. 1943.

Clinical observations by the writer emphasize the fact that the parasagittal symptom complex does not necessarily signify the presence of a meningioma.—A. C.

On the Classification of Meningiomas. A Survey of Ninety-Nine Cases in the Light of Existing Schemes. COURVILLE, C. B., and ABBOTT, K. H. [Coll. of Med. Evangelists and Los Angeles County Hosp., Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **6**:21-31. 1941.

The authors have grouped the meningeal tumors that they have studied into five unequivocal types; *i. e.*, syncytial, fibrous, transitional, angioblastic, and malignant, or sarcomatous, meningiomas. This classification is based primarily upon the morphology and the architectural arrangement of the tumor cells, secondary importance being placed on the formation of reticulin and collagen. The authors believe that the classification under the term "psammomatous meningiomas" is misleading and should be avoided since psammoma bodies are regressive in character, play no recognized part in the neoplastic process, and are found in many varieties of meningiomas.—A. C.

Neuro-Gliogenic Tumors of the Central Nervous System. Report of Two Additional Cases of Ganglioglioma of the Brain. COURVILLE, C. B., and ANDERSON, F. M. [Los Angeles County Hosp. and Coll. of Med. Evangelists, Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **6**:154-176. 1941.

In addition to the description of 2 new cases of neurogenic tumors of the brain, the paper includes a critical survey of similar cases found in the literature, and considerations regarding the proper classification of these tumors. Gangliogliomas (Ewing), are most commonly found arising from the floor of the third ventricle and from the centrum of the temporal lobe. The essential neoplastic cells are both of ganglionic and neuroglial origin. The presence of neoplastic ganglion cells in the reticular portions of the tumor should serve to identify it definitely as a ganglioglioma.—A. C.

A Case of Epidermoid Tumor of the Spinal Cord. Review of Literature of Spinal Epidermoids and Dermoids. CRAIG, R. L. [Duke Sch. of Med., Durham, N. C.] *Surgery*, **13**:354-367. 1943.

A review of the literature on spinal dermoids and epidermoids with a report of a case of the latter.—W. A. B.

Sphincter Disturbances Appearing Simultaneously with Weakness of Limbs in Compression of the Spinal Cord. FRIEDMAN, A. P. [Los Angeles County Hosp., Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **6**:73-76. 1941.

A report of two cases of extramedullary tumors of the spinal cord. One tumor developed in a patient with subacute leukemia and was classified as a chloroma; the other was not neoplastic.—A. C.

Osteochondroblastic Meningioma of the Left Cerebellar Hemisphere. FREIMAN, I. S., and FICARRA, B. J. [Kings County Hosp., Brooklyn, N. Y.] *Arch. Path.*, **35**: 900-905. 1943.

A case report.—J. G. K.

Removal of a Large Intraventricular Brain Tumour. HEBURN, H. H. [Provincial Mental Hosp., Edmonton, Canada] *Canad. M. A. J.*, **46**:477-478. 1942.

Removal of the tumor, which weighed 115 gm., was followed by rapid and uneventful recovery except for a left homonymous hemianopsia.—A. C.

Astrocytoma of the Cerebrum. HECHT, E. B. [Veterans' Administration, Legion, Tex.] *M. Bull. Vet. Admin.*, **18**: 441-445. 1941-42.

A case report.—M. E. H.

Malignant Tumors of the Brain. LOVE, J. G. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**: 83-84. 1941.

This paper includes introductory remarks on malignant tumors of the brain and the presentation of a case of a child 2 years of age from whom a highly malignant tumor of the fourth ventricle was removed.—J. L. M.

The Problem of Brain Tumor in Psychiatric Diagnosis. MCINTYRE, H. D., and MCINTYRE, A. P. [Cincinnati, Ohio] *Am. J. Psychiat.*, **98**:720-726. 1942.

Illustrative cases are presented together with operative and encephalographic studies of patients in whom one or more examiners failed to recognize tumor as the cause of psychotic symptoms.—M. E. H.

Sympathoblastoma in a New Born: A Case Report. MOORE, M. R., and GILDERSLEEVE, G. H. [Norwich, Conn.] *Connecticut M. J.*, **7**:101-103. 1943.

A case report with a short discussion of the histology of the tumor.—M. E. H.

Problems in the Diagnosis of Brain Tumors. With Report of a Case. PESSIN, J. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Wisconsin M. J.*, **41**:669-672. 1942.

A case is presented in which a malignant tumor of the left hemisphere (posterior parietal area) was associated with mental symptoms suggesting a psychosis. The role of mental symptoms in the diagnosis and localization of brain tumors is discussed.—M. E. H.

Bilateral Acoustic Neurofibromas. Report of Case in Sixteen Year Old Patient with Operative Removal and Autopsy Examination. REEVES, D. L. [Univ. of Southern California, Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **6**:91-103. 1941.

The present case gave no evidence of other cerebral neurofibromatosis. The possible relationship between the single acoustic tumor and von Recklinghausen's disease is discussed.—A. C.

Neuroblastoma Sympatheticum. ROSENBLUM, P. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:227. 1942.

A report of 2 cases.—M. E. H.

Solitary Neurogenic Sarcoma of the Mesentery. Review of the Literature and Report of a Case. SHAPIRO, M. J., and HOROWITZ, M. [Michael Reese Hosp., Chicago, Ill.] *Am. J. Surg.*, **61**:132-135. 1943.

The first reported case of this type. The patient, a 62 year old male, died of metastases 21 months after removal of the tumor at the base of the mesentery.—W. A. B.

Three Cases of Undiagnosticated Brain Tumor. SLOCUM, Y. K. [Veterans' Administration, Los Angeles, Calif.] *M. Bull. Vet. Admin.*, **18**:424-426. 1941-42.

A report of 3 cases.—M. E. H.

Glioma of the Diencephalon in a Manic Patient. STERN, K., and DANCEY, T. E. [Montreal, Canada] *Am. J. Psychiat.*, **98**:716-719. 1942.

A case report with autopsy findings.—M. E. H.

Malignant Predominantly Cystic (Unilocular) Cerebral Tumor (Meningioma) with Alveolar and Reticulin-Forming Cells. TUTHILL, C. R., and MEREDITH, J. M. [Univ. of Virginia Sch. of Med., Charlottesville, Va.] *South. M. J.*, **36**:471-478. 1943.

A cystic tumor of the left temperoparietal region occurring in a 14 year old boy was removed, with good results continuing 41 months after operation.—W. A. B.

Tumor of the Spinal Cord, with Successful Surgical Removal. WARREN, V. C., and THURSTON, J. A. [Veterans' Administration, Washington, D. C., and Atlanta, Ga.] *M. Bull. Vet. Admin.*, **18**:452-454. 1941-42.

A case report.—M. E. H.

Glioblastoma Multiforme. Report of Three Cases. WILLIAMS, H. W., and BERWALD, W. P. E. [Highland Hosp., Rochester, N. Y.] *Am. J. Surg.*, **60**:447-449. 1943.

A report of 3 cases.—W. A. B.

EYE

The Eye in Adrenal Sympathicoblastoma (Neuroblastoma). Importance of Ocular Findings, with First Pathologic Report of Metastatic Tumor in Childhood. BOTHMAN, L., and BLANKSTEIN, S. S. [Univ. of Chicago Sch. of Med., Chicago, Ill.] *Arch. Ophth.*, **27**:746-761. 1942.

Four cases of adrenal sympathicoblastoma with ocular manifestations are reported. Ecchymosis of the eyelids was the most constant finding in all 4. Papilledema occurred in 2 cases. This tumor metastasizes to the globe *via* the blood stream, as demonstrated by the presence of tumor cells in the medium sized blood vessels of the choroid in 1 case.—E. C. R.

Rhabdomyosarcoma of the Orbit. CALHOUN, F. P., JR., and REESE, A. B. [Inst. of Ophth., New York, N. Y.] *Arch. Ophth.*, **27**:558-578. 1942.

Fourteen cases from the literature are reviewed, and 5 additional ones are reported. The symptoms, clinical course, and surgical treatment are discussed. The histologic recognition of this type of tumor is described and illustrated.—E. C. R.

Carcinoma of the Limbus. EVANS, S. D. [Pittsburgh, Pa.] *Arch. Ophth.*, **27**:1132-1134. 1942.

A case of squamous cell carcinoma, treated by radiation following surgical removal of the tumor.—E. C. R.

Psammoma of the Orbit. FRY, W. E., and DELONG, P. [Wills Hosp., Philadelphia, Pa.] *Am. J. Ophth.*, **24**:664-668. 1941.

A general discussion with a report of a case.—E. C. R.

Tumors of the Optic Nerve. Study of Thirteen Cases from Brazil. Diagnosis, Degree of Malignancy. Clinical Classification. Histopathologic Classification. Relation to von Recklinghausen's Disease. Methods of Surgical Treatment. GOMES, J. P. [São Paulo, Brazil] Translated by PERERA, C. A. *Am. J. Ophth.*, **24**:1144-1169. 1941.

A general discussion based on 13 cases occurring in Brazil that had come to the author's attention.—E. C. R.

Idiopathic Multiple Hemorrhagic Sarcoma (Kaposi). Report of an Unusual Case in Which the Initial Lesion was on the Eyelid. GRAHAM, T. N. [New York Eye and Ear Infirmary, New York, N. Y.] *Arch. Ophth.*, **27**:1188-1192. 1942.

A case report in which the initial lesion was on the eyelid. Late widespread metastasis occurred.—E. C. R.

Primary Malignant Melanoma of the Choroid. HARLOWE, H. D. [Lenont-Peterson Clinic, Virginia, Minn.] *Minnesota Med.*, **25**:366-368. 1942.

A case of primary malignant melanoma of the choroid with metastases is reported. Callender's classification of melanosarcoma is discussed. Attention is called to the variation in time that may elapse between the primary lesion and the development of metastases. Earlier diagnosis is the only way now known for improving the prognosis.—J. L. M.

Bilateral Metastasis to the Eye Following Carcinoma of the Breast. JENSEN, A. F. [Grand Forks, N. D.] *Am. J. Ophth.*, **24**:63-66. 1941.

About 70% of metastatic carcinomas of the eye follow carcinoma of the breast. Report of a case.—E. C. R.

Retinal Tuberous Sclerosis (Bourneville's Disease). LOEWENSTEIN, A., and STEEL, J. [Glasgow, Scotland] *Am. J. Ophth.*, **24**:731-741. 1941.

The authors believe that this condition is a primary ectodermal malformation, and that the disturbance of balance of ectoderm and mesoderm leads secondarily to a mesodermal tumor. A case is reported and a general discussion of phacomas is given.—E. C. R.

Transcranial Removal of Intra-Orbital Tumors. Love, J. G. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:409-410. 1941.

Many intraorbital tumors can be removed satisfactorily by means of an anterior approach. Other intraorbital tumors, chiefly those situated behind the eyeball, are best removed from above through a transcranial approach. The latter technic provides excellent exposure of the orbital contents and permits accurate visualization of the tumor and surrounding structures. Adequate exposure is essential if the surgeon is dealing with angiomatous lesions, which have a tendency to bleed profusely. Visualization of the optic nerve enables the surgeon to prevent additional trauma to an already injured vital structure. In addition to the case presented in detail, the author has removed intraorbital tumors in 5 other instances. No deaths have occurred in this group of patients, and the results have been satisfactory.—J. L. M.

Treatment of Retinoblastoma (Retinal Glioma) Surgically and by Irradiation. Martin, H., and Reese, A. B. [Memorial Hosp., and Inst. of Ophth., New York, N. Y.] *Arch. Ophth.*, **27**:40-72. 1942.

This report is based on 23 patients observed at the Memorial Hospital. The management of unilateral and bilateral retinoblastomas together with their many complications is fully discussed. Enucleation of blind eyes is advised, and radiation therapy intended to control the disease and conserve useful vision is described in detail. Pathologic observations, with illustrations, are reported on eyes enucleated after receiving radiation.—E. C. R.

Primary Sarcoma of the Iris (Malignant Melanoma). A Report of Three Cases. McKee, S. H. [Montreal Gen. Hosp., Montreal, Canada] *Arch. Ophth.*, **28**: 197-204. 1942.

A general discussion with description of 3 new cases.—E. C. R.

Lymphosarcoma of the Lacrimal Gland. Report of a Case with Giant Lymph Follicle Hyperplasia. Perera, C. A. [Inst. of Ophth., New York, N. Y.] *Arch. Ophth.*, **28**:522-529. 1942.

A general discussion with report of a case.—E. C. R.

Orbital Tumors and Their Surgical Treatment. Part I. Reese, A. B. [Inst. of Ophth., New York, N. Y.] *Am. J. Ophth.*, **24**:386-394. 1941.

Orbital Tumors and Their Surgical Treatment. Part II. Reese, A. B. [Inst. of Ophth., New York, N. Y.] *Am. J. Ophth.*, **24**:497-502. 1941.

A general discussion of tumors and tumor-like masses in the orbit. The first part deals with tumors that are primary in the orbit; the second part with tumors adjacent to it.—E. C. R.

Primary Orbital Melanoma. Case Report with Review of the Literature. Rottino, A., and Kelly, A. S. [St. Vincent's Hosp., New York, N. Y.] *Arch. Ophth.*, **27**: 934-949. 1942.

A report of a case with concise summaries of 11 cases collected from the literature. Other instances are listed, and a good bibliography is added.—E. C. R.

The Surgical Correction of Neurofibromatosis (Plexiform Neuromata) about the Orbit. Spaeth, E. B. [Univ. of Pennsylvania, Philadelphia, Pa.] *Virginia M. Monthly*, **68**:630-642. 1941.

A series of 11 cases of neurofibromatosis about the orbit is presented to illustrate the surgical procedure necessary for correction.—M. E. H.

Female Genital Tract

Symposium on Gynecologic Cancer. I. General Consideration of Cancer of the Female Genitalia. Adair, F. L. [Univ. of Chicago, Chicago, Ill.] *Western J. Surg.*, **50**:433-438. 1942.

A general review of the incidence of cancer of the female genitalia and of the breast with some suggestions for improving the timing and quality of diagnostic and therapeutic services.—M. E. H.

Two Cases of Primary Carcinoma of the Fallopian Tube. Baker, J. O., and Blais, A. [Edmonton Gen. Hosp., Edmonton, Canada] *Canad. M. A. J.*, **46**:67-68. 1942.

A report of 2 cases in which surgical removal of the tumor (in 1 case by complete hysterectomy) was followed by apparent cure, the patients showing no recurrence 15 and 5 years after operation respectively.—A. C.

Thecoma of Ovary. Barnes, J. *Proc. Roy. Soc. Med.*, **36**:364. 1943.

Description of a case.—E. L. K.

The Direction of the Therapeutic Effort in Cases of Carcinoma of the Uterine Cervix. Bowing, H. H. [Mayo Clinic, Rochester, Minn.] *M. Clin. North America*, **25**:885-903. 1941.

Surgical treatment, radium therapy, and roentgen therapy, either singly or in combination, are the best procedures known at present for the treatment of carcinoma of the uterine cervix. This is equally true for adequate treatment of primary and secondary lesions and for the treatment of initial and late associated complications as they arise.

For a proper diagnosis, the history, palpation, bimanual rectoabdominal palpation, inspection, and biopsy should be included. The differential diagnosis is more tedious, since not every carcinoma occurring in the region of the lower uterine segment and vaginal vaults is a primary carcinoma of the uterine cervix. A careful follow-up system is necessary, since most of the early secondary lesions are symptomless in onset.—J. L. M.

Bilateral Ovarian Dermoid Cysts Complicating Pregnancy. Bowles, H. E. [Honolulu, T. H.] *West. J. Surg.*, **50**:78-81. 1942.

The author adds 5 cases to the 47 already reported in the literature and discusses the treatment of choice.—M. E. H.

Carcinoma of the Fundus of the Uterus. Brindley, G. V. [Scott and White Hosp., Temple, Tex.] *Ann. Surg.*, **114**:90-100. 1941.

One hundred and ten patients were studied. Abnormal vaginal bleeding was found to be the primary and most constant symptom. Diagnostic curettage, with microscopic confirmation of disease before selection of the type of treatment, is stressed. Treatment may be by irradiation, or surgery, or their combination. The highest

percentage of 5 year cures was obtained by the combined treatment.—M. R. D.

Carcinoma of the Cervical Stump. Cantril, S. T., and Bushke, F. [Swedish Hosp., Seattle, Wash.] *West. J. Surg.*, **50**:454-457. 1942.

The incidence and prognosis of cancer of the cervical stump should not make total hysterectomy imperative in every case in which removal of the corpus uteri is required, if enough attention is given to the condition of the cervix before subtotal hysterectomy.—M. E. H.

Some "Don'ts" in the Treatment of Carcinoma of the Corpus. DeCosta, E. J. [Chicago, Ill.] *West. J. Surg.*, **50**:452-453. 1942.

Illustrative examples emphasize the necessity of a complete and thorough general physical examination as well as the local examination and curettage, before major surgery is undertaken.—M. E. H.

Errors in the Treatment and Management of Carcinoma of the Cervix. Diddle, A. W. [State Univ. of Iowa, Iowa City, Iowa] *West. J. Surg.*, **50**:449-451. 1942.

Case summaries indicate a variety of representative examples of mismanagement in the treatment of early carcinoma of the cervix.—M. E. H.

Solid Ovarian Tumors. Dockerty, M. B. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**: 104-107. 1941.

Eighty per cent of ovarian tumors are cystic, and the majority of cystic tumors are benign. Twenty per cent of ovarian tumors are solid, and about two-thirds of the latter are malignant. Another important feature distinguishing ovarian tumors of the solid type is that 15% of these neoplasms produce hormones and give rise to peculiar clinical symptoms on the basis of altered physiologic processes. The clinical, pathological, and physiological features of some 500 solid ovarian neoplasms studied from the files of the Clinic are correlated in several tables. Photographs of histological sections of the neoplasms are included.—J. L. M.

Carcinoma of the Cervix: Time Lost Before Treatment. Hoge, R. H. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **69**:200-203. 1942.

The time elapsing before treatment in a series of 220 cases of carcinoma of the cervix is analyzed, and suggestions are offered for diminishing the delay.—M. E. H.

Hydatidiform Mole and Chorioepithelioma. A Comparison of Two Consecutive Five-Year Studies. Holman, A. [Portland, Oreg.] *West. J. Surg.*, **50**:319-326. 1942.

As a result of these two 5 year studies, the author feels that the ideal treatment for hydatidiform mole is thorough curettage of the uterus followed by frequent biologic pregnancy tests. If chorionepithelioma develops, hysterectomy is the procedure of choice. The only indication for removal of the ovaries is their involvement by the growth.—M. E. H.

Mesonephroma of the Ovary. Jensik, R. J. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:344-345. 1943.

A case report with the histological findings is presented. A basis for classification of these tumors is given in the discussion.—M. E. H.

An Improved Radical Technique for Carcinoma of the External Genitalia in the Female. Johnston, H. W. [Toronto, Canada] *Canad. M. A. J.*, **46**:230-233. 1942.

The lymphatic drainage of the external genitals is quite rich, with abundant anastomosis, so that a unilateral lesion can readily be propagated to both inguineal areas. An operative technic has been devised in which the superficial group of inguinal nodes is removed by the excision of a T-shaped mass of fat, while the deep seated inguinal nodes are removed after exposure through the division of Gimbernat's ligament. Eighteen drawings illustrate each step of the procedure.—A. C.

Radiation and Surgery in the Management of Ovarian Carcinoma. Keith, D. Y. [Louisville, Ky.] *South. M. J.*, **36**:490-494. 1943.

Discussion of 6 cases. The treatment advocated consists of removal of both ovaries, and irradiation of the pelvis by radium placed in the uterine canal as well as by external irradiation.—W. A. B.

Arrhenoblastoma of the Ovary. Krock, F., and Wolferman, S. J. [Fort Smith, Ark.] *Ann. Surg.*, **114**:78-89. 1941.

The final follow-up on a previously reported case of arrhenoblastoma of the ovary is presented. Several theories are discussed in an attempt to correlate physiologic manifestations with morphology. Reported cases are tabulated, and evidence is brought forth to support the conclusion that from a purely pathologic standpoint arrhenoblastoma may represent one-sided teratoma.—M. R. D.

Cystic Ovarian Tumors. MacCarty, W. C., Sr. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**: 101-103. 1941.

From a practical standpoint all cystic ovarian tumors are relatively benign except the solid carcinomatous cystadenomas. Death rarely follows complete removal of the papillary cystadenoma despite the serious appearance at exploration. Surgeons should be aware of the clinical nature of these tumors and be prepared to remove them since they will produce implants, ascites, emaciation, and death if not eradicated.—J. L. M.

Gynecological Malignant Disease. Some Problems in Treatment. McKelvey, J. L. [Univ. of Minnesota, Minneapolis, Minn.] *Minnesota Med.*, **24**:433-438. 1941.

This paper reviews the problems that are met in the handling of gynecological malignant conditions. The following points are discussed: (1) hospitalization of the patient undergoing radiation therapy in the lower abdomen, (2) justification of massive dose technics, (3) order of x-ray and radium treatments, (4) desirability of preliminary biopsy, (5) control of radiation dosage and its division, (6) origin of squamous cell carcinoma of the cervix, (7) significance of carcinoma of the cervix in young women, and (8) carcinoma of the body of the uterus.—J. L. M.

Ovarian Tumors Complicating Pregnancy. MacKenzie, R. A. [Asbury Park, N. J.] *J. M. Soc. New Jersey*, **39**:587-589. 1942.

Four cases are reported, illustrating the management of choice at various stages of pregnancy.—M. E. H.

Urinary Tract Pathology Associated with Carcinoma of the Cervix. Markowitz, I., and Katz, J. D. [Jersey City, N. J.] *J. M. Soc. New Jersey,* **39**:373-375. 1942.

In cases of carcinoma of the cervix, careful watch must be maintained on the urinary tract as the mortality rate due to pathologic conditions in this location is high (60 to 70%). The high mortality may be due to involvement by the carcinoma itself or indirectly to irradiation therapy.—M. E. H.

Cancer of Cervix and Fundus Uteri. Maxfield, J. R. [Parkland Hosp., Dallas, Tex.] *Southwestern Med.,* **26**:154-157. 1942.

This paper presents a review of the diagnosis and treatment of this condition. The author favors radiation therapy, either alone or combined with surgery, rather than surgery alone. Cancer of the uterus can be satisfactorily controlled if an early diagnosis is made and prompt, adequate treatment is given.—J. L. M.

The Management of Carcinoma of the Cervix Uteri. Mooney, B. R. [Winnipeg Gen. Hosp., Winnipeg, Canada] *Canad. M. A. J.,* **45**:521-524. 1941.

The paper is based on the study of 115 patients treated at the hospital between 1937 and 1940. In the great majority of cases, the disease was in an advanced stage when the patient was first seen by the physician. Often, delay in diagnosis because of the failure to see a physician when the first symptoms appeared was due admittedly to the fact that the patient felt she could not afford the cost of the treatment.

Abnormal vaginal bleeding is by far the most common and earliest warning of cancer of the cervix. Modalities in treatment with radium and x-radiations, depending on the clinical stage of the disease, are discussed.—A. C.

A Case of an Infiltrating Hydatiform Mole. Moss, L. D., and Wintermantel, J. A. [St. Francis Hosp., Olean, N. Y.] *Am. J. Clin. Path.,* **13**:267-270. 1943.

Positive Friedman tests persisting after the removal of intrauterine hydatidiform moles led to subsequent hysterectomy, with disclosure of a small, infiltrating, intramural hydatidiform mole.—J. G. K.

Present Day Concepts of Cancer of the Cervix. Rawls, J. L. [Norfolk, Va.] *Virginia M. Monthly,* **69**:249-252. 1942.

The results of treatment by radium and x-ray in 350 patients with carcinoma of the cervix are reported. An analysis of the replies to a questionnaire on methods of treatment of carcinoma of the cervix in 28 clinics in the United States and Canada is briefly presented.—M. E. H.

Carcinoma of Cervix Uteri; Wertheim's Hysterectomy; Tuberculosis of Regional Pelvic Lymphatic Glands. Rivett, L. C., and Bottomley, J. *Proc. Roy. Soc. Med.,* **36**:361-362. 1943.

Description of a case.—E. L. K.

Struma Ovarii. A Report of Two Cases. Sailer, S. [Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio] *Am. J. Clin. Path.,* **13**:271-277. 1943.

One of the lesions had the appearance of a thyroid adenoma; the other was of the colloid goiter type.—J. G. K.

Diagnosis and Treatment of Ovarian Tumors Presenting Endocrine Manifestations. Scheffey, L. C. [Jefferson Med. Coll., Philadelphia, Pa.] *M. Clin. North America,* **26**:1817-1830. 1942.

Nonneoplastic enlargements of the ovary are in the nature of either follicle or corpus luteum cysts. They represent a physiologic alteration that may or may not give rise to functional disturbance of an endocrine nature. Treatment is rarely surgical unless complicating pelvic disease requires operative interference.

On the other hand certain neoplastic enlargements of the ovary cause striking endocrine manifestations, either of a feminizing or masculinizing nature. The former are from the granulosa cell and allied tumors, and the latter from arrhenoblastoma and adrenal-like growths. Treatment in these instances is entirely surgical.—J. L. M.

Recent Advances in the Pathology of Ovarian Tumors. Schulze, M. [Univ. of California, San Francisco, Calif.] *West. J. Surg.,* **50**:37-42. 1942.

On the basis of embryological development, the author discusses the so called "special" ovarian tumors: the granulosa cell tumor, the arrhenoblastoma, the closely related disgerminoma, and the theca cell tumor.—M. E. H.

Serous Adenofibromas and Cystadenofibromas of the Ovary. Scott, R. B. [Johns Hopkins Sch. of Med., Baltimore, Md.] *Am. J. Obst. & Gynec.,* **43**:733-751. 1942.

Fourteen cases of serous adenofibromas and cystadenofibromas of the ovary are reported. The tumors consisted of a dense connective tissue matrix in which were embedded numerous small cystic spaces lined by compact, single-layered, cuboidal or low columnar, often ciliated, epithelium. In the gross the tumors were firm and solid. There was no constant associated pathologic condition in the pelvis. Pain was the common presenting complaint in 9 instances and in 6 of these cases it could definitely be related to pressure of the tumor. No endocrinologic importance could be attached to the tumors. Most of the patients were 40 years of age or older. Malignancy was not observed in any of the cases. It is suggested that these tumors be classified as a special type of epithelial tumor of the ovary under the subhead of serous cystadenomas.—A. K.

Uterine Fibroids with Pregnancy. Thompson, G. G. [Seattle, Wash.] *West. J. Surg.,* **49**:527-556. 1941.

The presence of uterine fibromyomas does not negate the possibility of pregnancy nor nullify the chance of its successful conclusion.—M. E. H.

Cancer of the Body of the Uterus. Watkins, R. E., and Neilson, D. R. [Univ. of Oregon Med. Sch., Portland, Oreg.] *West. J. Surg.,* **50**:17-31. 1942.

A review of 43 patients with respect to symptoms, physical findings, pathology of the disease, treatment, and follow-up, is presented.—M. E. H.

Implanted Epidermoid Carcinoma in the Pelvic Peritoneum. Report of a Case. Williams, C., and Bloom, N. [Richmond, Va.] *Virginia M. Monthly,* **69**:84-87. 1942.

A case is recorded of spontaneous rupture of a large dermoid cyst of the ovary, followed 9 months later by a large squamous cell (epidermoid) carcinoma in the cul-de-sac of Douglas.—M. E. H.

Neck

The Management of Lymph Nodes in the Neck—Metastatic from Carcinoma of the Mouth. Kennedy, R. H. [Post-Graduate Hosp., Columbia Univ., New York, N. Y.] *Ann. Surg.*, **114**:813-819. 1941.

The defeatist attitude toward treatment of cervical metastatic cancer must be overcome. Radiation therapy has contributed but little to the cure rate. Too frequently, surgical failures are due to the lack of knowledge of the lymph drainage areas and their inadequate dissection.—M. R. D.

The Treatment of Cervical Metastatic Cancer. Martin, H. [Memorial Hosp., New York, N. Y.] *Ann. Surg.*, **114**:972-985. 1941.

Five hundred consecutive cases of cervical metastatic cancer were studied according to the site of the primary lesions. Treatment by radiation, surgery, and their combination are comprehensively discussed and illustrated. End results are presented.—M. R. D.

Fibrochondroma in an Infant Four Weeks Old. Prewitt, L. H., and Brentan, E. [Ottumwa, Iowa] *Arch. Otolaryng.*, **36**:232-235. 1942.

A case report. The early age of the patient, 4 weeks at the time the tumor was surgically removed, makes this case of unusual interest.—M. E. H.

Intrathoracic Tumors—Lungs—Pleura

Observations on Intrathoracic Neoplasms. Alexander, J. [Univ. of Michigan, Ann Arbor, Mich.] *Ann. Surg.*, **114**:734-752. 1941.

Frequently there is dangerous delay in making the clinical and pathological diagnosis of intrathoracic neoplasms. Virtually every case of intrathoracic tumor should be promptly explored surgically, if there is no evidence of metastases or hopeless invasion of adjacent thoracic walls, and if the patient's general condition permits. Low operative mortality rate and uncomplicated convalescence depend upon the observance of certain special preoperative, operative, and postoperative principles.—M. R. D.

Pneumonectomy. Berry, F. B. [Columbia Univ., New York, N. Y.] *Ann. Surg.*, **114**:32-45. 1941.

A comprehensive discussion of the development of pneumonectomy and its application to various types of pulmonary disease. A comparison is given of the results of surgery for cancer of the stomach and of the lungs. Eighteen clinical cases in which pneumonectomy was done are summarized.—M. R. D.

Primary Pulmonary Carcinoma. Bondurant, A. J. [Veterans' Administration, Jefferson Barracks, Mo.] *M. Bull. Vet. Admin.*, **18**:386-393. 1941-42.

A short résumé on the incidence, diagnosis, and treatment of primary pulmonary carcinoma followed by a case report.—M. E. H.

Carcinoma of the Lung; A Review of 31 Proved Cases at the Philadelphia Naval Hospital. Fetter, F. [Med. Corps, U. S. Naval Reserve, Philadelphia, Pa.] *Ann. Int. Med.*, **18**:979-987. 1943.

A clinical discussion, emphasizing the frequency of the disease and the desirability of early bronchoscopy and exploratory thoracotomy in suspected cases.—J. G. K.

Tobacco Smoking and Cancer of the Lung. Grace, E. J. [Brooklyn, N. Y.] *Am. J. Surg.*, **60**:361-364. 1943.

Three cases of carcinoma of the lung are described, and the possible role of tobacco smoke in the etiology is discussed.—W. A. B.

Lobectomy for Benign Pulmonary Neoplasm. Kessler, I. [Veterans' Administration, Washington, D. C.] *M. Bull. Vet. Admin.*, **17**:396-397. 1940-41.

A case of benign angioma of the lung successfully treated by lobectomy.—M. E. H.

Carcinoma of the Lung with Pulmonary Tuberculosis. Martin, A., and Beaudet, E. A. [Veterans' Administration, Livermore, Calif.] *M. Bull. Vet. Admin.*, **17**:404-406. 1940-41.

A case report with necropsy findings.—M. E. H.

Benign Bronchial Adenoma: Report of a Peripheral Lesion of the Right Middle Lobe, Cured by Partial Lobectomy. Mayo, L. E. [Portsmouth, Va.] *Virginia M. Monthly*, **69**:550-554. 1942.

A tumor occurring in the periphery of the right middle lobe, of a type hitherto reported only in bronchi of the first and second order, and a favorable response to lobectomy is reported.—M. E. H.

Intracranial Metastasis as Early Evidence of Bronchial Carcinoma. Mitchell, H. C., and Werba, D. R. [Veterans' Administration, Hines, Ill., and Minneapolis, Minn.] *M. Bull. Vet. Admin.*, **18**:214-217. 1941-42.

A case report.—M. E. H.

A propósito de dos observaciones de metástasis óseas de carcinomas ignorados de pulmón. [The Occurrence of Bone Metastases in Two Unsuspected Carcinomas of the Lung.] Muscolo, D., and Bianchi, A. [Inst. de Radiología y Fisioterapia, Buenos Aires, Argentina] *Rev. ortop. y traumatol.*, **11**:281-290. 1942.

A report of 2 cases in which bone metastases were evident 2 and 7 months before pulmonary symptoms were manifest. Nine illustrations are appended.—M. D-R.

Neo de pulmón tratado por roentgenterapia ultra profuunda (600 k. v.). [Neoplasm of the Lung Treated by Ultra-Deep Roentgentherapy (600 kv.).] Roffo, A. E., Jr. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. de cáncer*, **18**:993-999. 1941.

Report of a case successfully treated.—M. D-R.

Endothelioma of the Pleura. With Report of Two Cases. Saccone, A., and Coblenz, A. [Metropolitan Hosp., Welfare Island, N. Y.] *Am. J. Clin. Path.*, **13**:186-207. 1943.

Four figures illustrate the 2 cases, which are reported in detail, together with a discussion of the literature.—J. G. K.

Bronchiogenic Carcinoma. Simons, E. J. [Swanville, Minn.] *Minnesota Med.*, **24**:438-442. 1941.

The author reviews bronchiogenic carcinoma under the following subtitles: (1) historical data, (2) incidence, (3) etiology, (4) pathology, (5) clinical considerations—symptomatology and physical signs, (6) differential diagnosis, (7) diagnostic aids—pleural effusion, sputum, roentgenology, and bronchoscopy, and (8) treatment—palliative measures, irradiation, and surgery.—J. L. M.

Tumors Occurring in the Region of the Pulmonary Apex. Further Observations with Report of Twelve Additional Cases. STEIN, J. J. [Veterans' Administration, Hines, Ill.] *Illinois M. J.*, **81**:21-29. 1942.

Case reports and further evidence to show that the majority of malignant tumors in the region of the thoracic inlet or pulmonary apex are carcinomas of the terminal bronchioles of the lung.—M. E. H.

Cellular Origin of Bronchial Adenoma. STOUT, A. P. [Coll. of Physicians and Surgeons, New York, N. Y.] *Arch. Path.*, **35**:803-807. 1943.

The peculiar cells with acidophilic granules called oncocytes or pyknocytes were demonstrated among the mucous and serous glands of adult human bronchi and their ducts. The relationship of these cells to the cells of bronchial adenoma is discussed, and it is considered possible that they may be the stem cells for tumors of this type.—J. G. K.

The Importance of Physical Examination in the Diagnosis of Primary Bronchial Carcinoma. VINSON, P. P. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **69**:631. 1942.

Suppression of breath sounds without impairment of percussion note is important in the diagnosis of primary bronchial carcinoma.—M. E. H.

Lipoma of Left Main Bronchus. Report of a Case and Review of Literature. VINSON, P. P., and PEMBLETON, W. E. [Med. Coll. of Virginia, Richmond, Va.] *Arch. Otolaryng.*, **35**:868-870. 1942.

The report deals with what is apparently the eighth case of lipoma of the bronchus to be recorded.—M. E. H.

GASTROINTESTINAL TRACT

Gastric Ulcer. The Significance of This Diagnosis and Its Relationship to Cancer. ALLEN, A. W., and WELCH, C. E. [Massachusetts Gen. Hosp., Boston, Mass.] *Ann. Surg.*, **114**:498-509. 1941.

This study deals with 277 patients in whom the original diagnosis was gastric ulcer. Thirty-nine, or 14%, proved to have cancer. The seriousness of this disease is stressed, and the clarification of ideas concerning management is urged. Evidence is presented that gastric ulcer is a surgical disease.—M. R. D.

Carcinoma of Oesophagus—Treated by Excision and Reconstruction of Ante-Thoracic Oesophagus. ALLISON, P. R. *Proc. Roy. Soc. Med.*, **36**:341-342. 1943.

Description of a case.—E. L. K.

Transduodenal Resection of Carcinoma of the Ampulla of Vater. BAUMGARTNER, C. J. [St. Vincent's Hosp., Los Angeles, Calif.] *West. J. Surg.*, **50**:250-253. 1942.

A case is reported with the operative technic employed. —M. E. H.

Primary Sarcoma of the Duodenum; Resection with Head of Pancreas by One-Stage Whipple Operation. BISGARD, J. D., and COCHRAN, R. M. [Omaha, Neb.] *Surgery*, **12**:388-389. 1942.

Microscopic examination showed the tumor to be lymphosarcoma. Death from recurrence took place 3 months after operation.—W. A. B.

Leiomyoma of the Stomach—A Case Report. BLOOM, N., and WILLIAMS, C. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **69**:627-629. 1942.

A case report.—M. E. H.

Carcinoma of the Esophagus: Report of a Case with Resection and Esophagogastrostomy. BRANTIGAN, O. C., and SHIPLEY, A. M. [Univ. of Maryland Sch. of Med., Baltimore, Md.] *Bull. Univ. Maryland School Med.*, **27**:165-171. 1943.

A successful esophageal resection with esophagogastrostomy is reported. The authors believe that the absence of postoperative suffering, the ability to eat normally and again to carry on a normal existence makes the operation worth while even though the patient should live only a few months.—J. L. M.

Progress in a Case of Adenocarcinoma of the Stomach, Grade 4, Treated by Subtotal Gastrectomy. CAMPBELL, D. C. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:45-48. 1941.

A case of adenocarcinoma of the stomach is presented to illustrate how a good result may be obtained in the face of many ominous prognostic signs including a history of short duration, achlorhydria, involvement of lymph nodes, involvement of the serosa, and carcinoma, grade 4 (Broders' classification). Almost 4 years have elapsed since operation without evidence of recurrence.—J. L. M.

Regional Lymphatic Metastases of Carcinoma of the Colon. COLLER, F. A., KAY, E. B., and MACINTYRE, R. S. [Univ. of Michigan, Ann Arbor, Mich.] *Ann. Surg.*, **114**: 56-67. 1941.

A study of the lymph nodes in 46 specimens of carcinoma of the colon was made by David and Gilchrist's modification of the method of Spalteholtz. The routes of spread of carcinoma by the lymph channels are illustrated and discussed. Factors influencing prognosis are summarized. —M. R. D.

Carcinoma of the Large Bowel, 1932-1941, Waterbury Hospital. COLLINS, J. O. [Waterbury, Conn.] *Connecticut M. J.*, **6**:365. 1942.

Since the general hospital of medium size does not offer sufficient material for all surgeons on its staff to perfect their technic in surgery of the colon and rectum, the suggestion is advanced that all cancers be handled according to anatomical group by different teams of surgeons. This would enable a surgical team to perfect a technic in a particular anatomical field, which should result in general improvement in cancer therapy in the general hospital.—M. E. H.

Carcinoma of the Colon: the Earliest Possible Stage of Clinical Recognition. CROMAR, C. D. L., BARGEN, J. A., and DIXON, C. F. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:641-643. 1941.

The authors' figures show that by present standards the earliest clinically recognizable carcinoma of the colon may be an advanced pathological growth. They also indicate that the future of any given case depends more on the grade of the lesion than on its physical properties.—J. L. M.

A Consideration of the Contraindications for Radical Operation in Cancer of the Rectum. DAVID, V. C., and GILCHRIST, R. K. [Chicago, Ill.] *Surgery*, **12**:310-314. 1942.

The contraindications for radical operation in 277 patients with carcinoma of the rectum were: liver metastases (40 cases), attachment to the base of the bladder (28 cases), and infiltrative attachment to the sacrum, prostate, or rectovaginal septum (23 cases).—W. A. B.

Lymphosarcoma of the Stomach with Perforation. Gastric Resection with Recovery. Doran, W. T., and Doran, W. T., Jr. [Bellevue Hosp., New York, N. Y.] *Am. J. Surg.*, **61**:136-137. 1943.

Perforation of a lymphosarcoma of the stomach was found at operation. Subtotal resection was done and x-ray therapy instituted (1,750 r). The patient was alive and apparently well 1 year later.—W. A. B.

Benign Tumors of the Stomach. Finesilver, E. M. [Cornell Univ. Med. Coll., New York, N. Y.] *Surgery*, **12**: 216-235. 1942.

A review of cases, 5 with polyps, 1 with a leiomyoma.—W. A. B.

The Use of Sulfanilylguanidine in Surgical Patients. Firor, W. M., and Jonas, A. F. [Baltimore, Md.] *Ann. Surg.*, **114**:19-31. 1941.

A presentation of the records of 12 patients who had received sulfanilylguanidine before resections of the colon. It is the impression that there were more *per primam* healings and smoother convalescence in complicated cases.—M. R. D.

The Billroth I Type of Operation for Carcinoma of the Stomach. Friedell, M. T. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:481-487. 1941.

The Billroth I operation (gastric resection and gastroduodenostomy) is the procedure of choice in the treatment of malignant lesions of the stomach when employed in favorable circumstances. It has the advantages of speed and less surgical manipulation, which are valuable factors in operations on elderly, debilitated persons, especially those who have small lesions in the lower end of the stomach. Applied correctly, it has offered a good chance for a 5 year cure (35%) with a low average hospital mortality rate (11%).—J. L. M.

Aseptic, Immediate Anastomosis Following Resection of the Colon for Carcinoma. Gibbon, J. H., Jr., and Hodge, C. C. [Pennsylvania and Bryn Mawr Hosps., Philadelphia, Pa.] *Ann. Surg.*, **114**:635-652. 1941.

One hundred and twenty cases of carcinoma of the colon proximal to the rectosigmoid are reviewed. This small series of cases combined with the reported statistics of MacFea, Wilkie, and Stone, and of McLanahan reveal the following: In 246 patients operated upon by the aseptic technic, the operative mortality was 14%; in 124 patients operated upon by exteriorization methods, it was 27%; in 72 patients undergoing an open anastomosis, it was 28%. These combined statistics appear to indicate that the aseptic, immediate anastomosis is the operation to be preferred.—M. R. D.

Carcinoma Metastases in Appendices Epiploicae. Gilchrist, R. K., and David, V. C. [Presbyterian Hosp., Chicago, Ill.] *Surgery*, **13**:574-577. 1943.

Lymph nodes in appendices epiploicae contained carcinoma in 5 surgically removed specimens of carcinoma of the rectum or sigmoid. Preparations injected with India ink demonstrated lymph channels on the antimesenteric border of the sigmoid that sometimes travelled 2 to 3 cm. lengthwise before turning to drain into the mesentery. These observations suggest that, for radical resection of the sigmoid for carcinoma, the colon should be divided at least 3 cm. from the margin of the neoplasm.—W. A. B.

Carcinoid (Argentaffin Cell Tumor) of the Vermiform Appendix. Ginsberg, S. T., Sollod, B. W., and Wilcox, E. A. [Veterans' Administration, Marion, Ind., Bay Pines, Fla., and Augusta, Ga.] *M. Bull. Vet. Admin.*, **18**:309-312. 1941-42.

A case report.—M. E. H.

Malignant Lesions of the Colon and Rectum: Operability and Prognosis. Gregg, R. O., and Dixon, C. F. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:657-659. 1941.

During the period from 1907 to 1938 inclusive, 9,632 patients with a diagnosis of malignant lesion of the colon or rectum were seen at the Mayo Clinic. Many irremovable lesions were treated by radium or roentgen rays; in a few instances the growth was fulgurated; a certain number of lesions were deemed inoperable; and some patients refused operation.

For 74% of the patients seen, surgical exploration was undertaken; for 68% of these resection was performed. On the remaining 32% of patients who underwent surgery, operations of a palliative nature, such as colostomy or a short circuiting operation were carried out. Palliative resections were frequently performed whenever relief could be accomplished and the patient's condition appeared to be fair or good, even though evidence of metastasis was found at exploration.

The high percentage (75%) of low grade lesions in this series accounts in part for the relatively favorable prognosis in such cases compared to that in cases of malignant lesions in some other parts of the body. Prognosis was also directly correlated with lymphatic involvement. The 5 year prognosis in cases of grade 4 lesions without metastasis was more favorable than in those in which the lesions were of histopathological grade 1 and metastasis to regional lymph nodes was present.—J. L. M.

Recurrent Carcinoma of the Colon: Report of Four Cases. Gregg, R. O., and Dixon, C. F. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:177-181. 1941.

The authors present 4 cases to illustrate the advisability of exploration in apparent recurrent carcinoma of the colon. If a patient thought to have such a recurrence is in good condition, they believe an exploratory procedure is indicated, since (1) a lesion which appears to be recurring carcinoma may be a benign condition, (2) the apparent recurrent tumor may be a new lesion and therefore merit as radical treatment as did the first malignant growth, and (3) removal of a locally recurring malignant tumor may be followed by many years of comfortable living for the patient or even permanent relief from the disease.—J. L. M.

Gastric Cancer. Horsley, G. W. [St. Elizabeth's Hosp., Richmond, Va.] *Virginia M. Monthly*, **69**:126-134. 1942.

The author stresses diagnosis as the important point in cancer of the stomach, as surgical excision is the only known method of cure.—M. E. H.

Malignant Lesions of the Stomach. Hunt, V. C. [Los Angeles, Calif.] *Southwestern Med.*, **26**:326-329. 1942.

The following topics are discussed in connection with carcinoma of the stomach: operability, diagnosis, gastric ulcer and malignancy, and surgical procedures. It is worthy

of emphasis that in accordance with present knowledge of carcinoma of this organ early gastric resection provides the only opportunity for cure.—J. L. M.

Surgical Management of Carcinoma of the Ampulla of Vater and of the Periampullary Portion of the Duodenum. HUNT, V. C. [Los Angeles, Calif.] *Ann. Surg.*, **114**:570-602. 1941.

The 124 cases successfully operated upon since Halsted's original case in 1898 are reviewed, and 4 new ones are added. Abstracts of 32 cases, not previously collected, are appended. Diagnosis, surgical procedures, operative mortality, and the results in general are discussed.—M. R. D.

Cancer of the Rectum: Preoperative and Postoperative Complications. JELKS, J. L. [Memphis, Tenn.] *South. M. J.*, **36**:467-471. 1943.

Personal recollections.—W. A. B.

Recurrent Carcinoma of the Rectum. JOHNS, F. S. [Richmond, Va.] *Ann. Surg.*, **114**:68-72. 1941.

Surgical treatment should be considered more frequently for recurrent carcinoma of the rectum. One case report is presented.—M. R. D.

Primary Carcinoma of the Jejunum. KARRAS, R. W. [Veterans' Administration, Dwight, Ill.] *M. Bull. Vet. Admin.*, **17**:402-403. 1940-41.

A case report with surgical and autopsy findings.—M. E. H.

Regional Lymphatic Metastases of Carcinoma of the Gastrointestinal Tract. KAY, E. B. [Univ. of Michigan, Ann Arbor, Mich.] *Surgery*, **12**:553-562. 1942.

Lymph nodes were obtained by the dissection of cleared specimens and examined microscopically. Lymph node metastases were found in 75.5% of 53 gastric carcinomas, 60.87% of 46 colonic carcinomas, and 64.2% of 53 rectal carcinomas. There was no relation between the duration of symptoms and the presence of lymph node metastases, but sessile neoplasms had a greater tendency to metastasize than the polypoid neoplasms. There was no relation between the size of the neoplasm and the presence of metastases.—W. A. B.

Acute Perforation of Lymphosarcomatous Ulcer of the Stomach. Report of a Case. KOUCKY, J. D., BECK, W. C., and ATLAS, J. [Cook County Hosp., Chicago, Ill.] *Ann. Surg.*, **114**:1112-1116. 1941.

A case report of lymphosarcoma of the stomach with perforation.—M. R. D.

Carcinoid Tumors of the Stomach. LEMMER, K. E. [Univ. of Wisconsin Sch. of Med., Madison, Wis.] *Surgery*, **12**:378-382. 1942.

A case report.—W. A. B.

Total Gastrectomy for Extensive Carcinoma of the Stomach: Report of Case. LYNCH, R. C., and PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:653-656. 1941.

A case report.—J. L. M.

Malignant Lesions of the Right Portion of the Colon. MAYO, C. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:67-69. 1941.

This is a brief review of a study of 885 cases of malignancy of the cecum and ascending colon in which operation was performed at the Clinic from 1907 to 1938. In 67% of these cases, resection was performed with a view to cure.

The author states that any person who has anemia of undetermined cause should not be dismissed without roentgenologic examination of the colon. In 15% of the cases in this series, appendectomy had been performed after the onset of symptoms attributable to the lesions in the right portion of the colon. Only a little more than 2% of the patients were less than 30 years old. It is suggested therefore, that whenever operation is performed for appendicitis on a patient who is more than 30 years of age, an incision be made that is large enough to permit examination of the right portion of the colon also.

As a result of this study the author favors the one stage resection of the right portion of the colon, as this usually means a lower hospital mortality rate and lower morbidity. It also removes the carcinoma from the body at an earlier time than any multiple stage type of resection. In addition the one stage resection has the advantage of being a more economical method of dealing with the situation, a factor which is of prime importance to most of those suffering from this condition.—J. L. M.

One Stage Combined Abdominoperineal Resection for Malignant Tumors of the Rectum, Rectosigmoid, and Lower Part of Sigmoid. MAYO, C. W. [Mayo Clinic, Rochester, Minn.] *Surg., Gynec. & Obst.*, **76**:649-654. 1943.

A clinical discussion.—J. G. K.

Pregnancy Complicating Carcinoma of the Rectum or Carcinoma of the Rectum Complicating Pregnancy. MAYO, C. W., and HUNT, A. B. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:513-516. 1941.

The authors report a pregnant patient in whom, after removal of a carcinoma of the rectum, the pregnancy was successfully terminated 5 months after the first operation by an elective low cervical cesarean section.—J. L. M.

Prognosis Following Palliative Resection for Carcinoma of the Sigmoid. MAYO, C. W., and MILLER, J. M. [Mayo Clinic, Rochester, Minn.] *Minnesota Med.*, **24**:84-86. 1941.

The observations concerning a group of 55 patients, for whom palliative resection of the sigmoid for inoperable carcinoma was performed, have been reviewed. Comparison of these observations with those made on a group of patients subjected to colocolostomy, reveals a slightly lower primary surgical mortality and a shorter postoperative duration of life for members of the latter group.—J. L. M.

End-to-End Ileocolostomy: Indications for, and Evaluation of, in Resection of Right Portion of Colon in One Stage for Malignant Lesions. MAYO, C. W., and SCHLICKE, C. P. [Mayo Clinic, Rochester, Minn.] *Surgery*, **12**:716-728. 1942.

A review of the various procedures used in dealing with carcinoma of the right side of the colon. Resection in one stage with end-to-end ileocolostomy was done in 96 cases with 18 deaths. In 42 cases complementary enterostomy was done (mortality 28.6%); in 54 cases enterostomy was not done (mortality 11.1%).—W. A. B.

Metastasizing Argentaffine Tumor of the Cecum in a Case of Multiple Colonic Malignancies. Mayo, C. W., and Wilson, W. D. [Mayo Clinic, Rochester, Minn.] *Minnesota Med.*, **24**:178-179. 1941.

It is generally conceded at present that the so called carcinoid tumors arise from the Kultschitzky cells, which are found in the base of the crypts of Lieberkühn. The ability of the cells of this tumor to reduce silver provides the surgeon with a positive means of diagnosis, although if ordinary hematoxylin and eosin preparations are used the arrangements of the cells in groups will be characteristic enough. On gross examination the surgeon may gain an impression of the nature of the tumor by the yellow color that, in most instances, is present. The malignancy of carcinoid tumors becomes more obvious as more cases are reported.—J. L. M.

Intrathoracic Esophagojejunostomy for Total Gastrectomy with Lower Esophagectomy for Carcinoma. Meyer, H. W. [New York, N. Y.] *Surgery*, **12**:115-127. 1942.

A report of successful resection of the stomach and lower 5 cm. of the esophagus with death due to carcinomatosis 5 months later.—W. A. B.

Carcinoma of the Colon and Rectum. Miller, G. [McGill Univ., Montreal, Canada] *Canad. M. A. J.*, **46**:565-569. 1942.

The paper is a survey of the present status of carcinoma of the colon and rectum. The slightest uneasiness in the left lower quadrant, a slight pain, a change in bowel habit, especially in patients over forty, suggests the possibility of carcinoma and calls for a thorough investigation. The greatest single cause of mortality in large bowel resection seems to be infection. It is felt that chemotherapy and improvements in surgical technic may reduce the mortality to about 5%, instead of the 70% of only a decade ago.—A. C.

Carcinoma of the Rectum. Nesselrod, J. P., Garner, J. M., Christopher, F., and Jennings, W. K. [Northwestern Univ. Med. Sch., Chicago, Ill.] *Illinois M. J.*, **81**:316-321. 1942.

Diagnosis and operative treatment as well as preoperative and postoperative care are reviewed.—M. E. H.

Leiomyoma of the Small Intestine. Case Report. Patterson, D. C., and Geer, W. A. [Bridgeport Hosp., Bridgeport, Conn.] *Connecticut M. J.*, **7**:168-169. 1943.

A case report.—M. E. H.

Total Gastrectomy (the Report of a Case). Peikoff, S. S. [Winnipeg, Canada] *Canad. M. A. J.*, **45**:407-410. 1941.

Total gastrectomy was indicated because of ulcer and infiltrating adenocarcinoma near the cardia. Recovery was uneventful. A year and a half after the operation the patient had gained 15 pounds and was well. Physiologically it is interesting to note that, although the stomach was absent, the patient experienced a feeling of hunger and had a fair appetite.—A. C.

Advanced Carcinoma of the Colon and Rectum. Phillips, R. B., and Dixon, C. F. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:182-187. 1941.

One of the most important palliative procedures for obstructive carcinoma of the colon and rectum, in the presence of metastasis, is undoubtedly the establishment of colostomy; in some such cases the growths can be removed, or treated with radium and fulguration, or with fulguration alone, depending on circumstances. The authors present several cases of extensive carcinoma of the colon in which operations have been performed.—J. L. M.

Carcinoma of the Stomach. Priestley, J. T. [Mayo Clinic, Rochester, Minn.] *Minnesota Med.*, **24**:81-84. 1941.

At present the only hope for cure of carcinoma of the stomach resides in surgical removal of the growth following a sufficiently early establishment of the diagnosis. The diagnosis of carcinoma of the stomach is established by means of the following: appreciation of the early symptom complex of this lesion, a carefully elicited history, an easily aroused suspicion of gastric carcinoma, and the insistence on roentgenologic examination by a competent specialist in this field in any case in which the presence of a malignant lesion is faintly suspected. Of patients who survived gastric resection, 29% were alive 5 years after operation.—J. L. M.

Tratamiento electroquirúrgico del cáncer del recto. [Electrosurgical Treatment of Cancer of the Rectum.] Roffo, A. H., and Carranza, F. [Inst. de med. exper. para estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **18**:1027-1082. 1941.

A study based on 926 cases. The authors do not favor radiation in cases of cancer of the rectum, but regard electrosurgery as the method of choice. Of 261 patients treated by the latter method, 5 year cures were obtained in 50% with only 2% operative mortality. Thirty-eight illustrations are appended.—M. D-R.

Pedunculated Tumors of the Esophagus. Samson, P. C., and Zelman, J. [San Joaquin Gen. Hosp., Stockton, Calif.] *Arch. Otolaryng.*, **36**:203-211. 1942.

A case report and a survey of 26 cases previously recorded form the basis of this study. The tumors occur predominantly in men after the fifth decade of life. Malignant change is uncommon. Cures are reported following removal in 8 cases: in 2 by external esophagotomy, and by endoscopic means in 6. It is the opinion of the authors that endoscopic removal is the superior method.—M. E. H.

Thoracic Esophagectomy for Cancer. Report of Two Successful Cases. Santy, P., Ballivert, M., and Berard, M. [Lyons, France] *J. Thoracic Surg.*, **12**:397-431. 1943.

Of 28 patients with carcinoma of the esophagus, 22 had obviously inoperable lesions. Four of the remaining 6 were subjected to exploratory thoracotomy but in only 2 was it feasible to attempt removal of the tumors. The latter were situated in the upper two-thirds of the esophagus. The operative technic is discussed in detail. A right transpleural approach is advocated because of lessened chance of establishing bilateral pneumothorax. Esophagectomy was preceded by gastrostomy and exploratory laparotomy. At the time of report the patients were well 7 and 6 months respectively after resection.—E. E. S.

Some Problems in the Management of Cancer of the Rectum. Silvers, H. I. [Atlantic City Hosp., Atlantic City, N. J.] *J. M. Soc. New Jersey*, **39**:323-327. 1942.

A review of 100 cases of carcinoma of the rectum with the various operative procedures used in 67 patients submitting to operation.—M. E. H.

Papillary Carcinoma of the Duodenum. Tremor, V. F. [Veterans' Administration, Indianapolis, Ind.] *M. Bull. Vet. Admin.*, **18**:313. 1941-42.

A case report.—M. E. H.

Primary Carcinoma of the Appendix Resembling Carcinoma of the Colon. Uihlein, A., and McDonald, J. R. [Mayo Clinic, Rochester, Minn.] *Surg., Gynec. & Obst.*, **76**:711-714. 1943.

Five cases are reported.—J. G. K.

Cancer of the Esophagus. Clinical Observations in a Series of 930 Cases. Watson, W. L. [Memorial Hosp., New York, N. Y.] *Connecticut M. J.*, **6**:959-961. 1942.

The author discusses the problem of cancer of the esophagus under several headings: anatomy, micropathology, metastases, symptomatology, etiology, and treatment. It is of interest that multiple cancer occurred in this series 16 times and the second primary growth was intraoral in 87.5% of these, leading to the suspicion that the same predisposing factors were responsible for the production of both cancers. Treatment consisted of radiation or surgery.—M. E. H.

Total Gastrectomy and Partial Esophagectomy for Carcinoma: Report of Successful Operation for Oldest Known Patient. Waugh, J. M., and Giffin, L. A. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:363-365. 1941.

As far as the authors were able to ascertain from the literature, their 72 year old patient represents the oldest person to undergo total gastrectomy successfully. The fact that the esophagus was mobile and long enough to permit successful resection of the lower 1½ inches together with the stomach, suggests that in selected instances many patients suffering from carcinoma involving the cardia and lower part of the esophagus, with esophageal dilatation and obstruction, for whom operation has been refused in the past, may undergo this procedure with reasonable hope of success.—J. L. M.

Carcinoid Tumor of the Cecum. Waugh, J. M., and Snyder, J. M. [Mayo Clinic, Rochester, Minn.] *Ann. Surg.*, **114**:151-152. 1941.

Only 12 cases of carcinoid tumors of the colon have been reported. A case report is presented involving a tumor arising in the cecum. These tumors are usually found at laparotomy, are relatively benign, and may be locally resected.—M. R. D.

Ulcerated Hemorrhagic Leiomyosarcoma of the Stomach: Report of a Case Nine Months after Partial Gastrectomy. White, R. R., and Walters, W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:378-381. 1941.

Although differential diagnosis of sarcoma of the stomach is difficult clinically, if a history of a large epigastric mass that has been present a long time is elicited from a patient who has suffered little loss of weight and little general debility, the presence of a tumor of this type should be suspected. A rather severe secondary anemia may often be a prominent feature in such cases. Roentgenologic examination is not conclusive in the differentiation. An illustrative case is presented.—J. L. M.

Carcinoma of the Cecum: Report of a Case. Wilcox, L. E., and Clagett, O. T. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clinic*, **16**:534-536. 1941.

All three physicians who saw the patient concurred in the diagnosis of acute appendicitis, and immediate operation was advised. An exploration, carried out through a primary McBurney incision, revealed a large carcinoma of the cecum. This was removed; the postoperative course was uneventful.—J. L. M.

Adenomatosis of the Duodenum. Report of a Case. Wolfer, J. A. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:261-262. 1942.

A case report.—M. E. H.

Primary Anastomosis or Exteriorization and Resection of the Cancerous Colon. Woolf, M. S. [Univ. of California Hosp. Sch., San Francisco, Calif.] *West. J. Surg.*, **50**:458-462. 1942.

A discussion of the surgical method of choice and suggestions for preoperative and postoperative care are offered with the view of further reducing the operative mortality. —M. E. H.

Liver

Surgical Excision of Primary Tumor of Liver (Hamartoma) in Infant Seven Months Old with Recovery. Benson, C. D., and Penberthy, G. C. [Detroit, Mich.] *Surgery*, **12**:881-886. 1942.

A case report.—W. A. B.

Primary Carcinoma of the Liver. Berns, R. S. [Veterans' Administration, Danville, Ill.] *M. Bull. Vet. Admin.*, **18**:211-213. 1941-42.

A case report.—M. E. H.

Carcinoid Tumor of the Gallbladder. Bosse, M. D. [Western Pennsylvania Hosp., Pittsburgh, Pa.] *Arch. Path.*, **35**:898-899. 1943.

This appears to be the third reported case of carcinoid tumor of the gall bladder.—J. G. K.

Case of Hepatomegaly (Nature Undetermined). Ledlie, R. C. B. *Proc. Roy. Soc. Med.*, **36**:357-358. 1943.

Description of a case.—E. L. K.

Carcinoma of the Gall Bladder: Study of Sixty Cases. Mattson, H. [Univ. of Minnesota, Minneapolis, Minn.] *Minnesota Med.*, **25**:985-988. 1942.

Carcinoma of the gall bladder is discussed under the following headings: age and sex, symptoms, roentgen diagnosis, and pathology. The author finds that the clinical picture will fit many cases of benign biliary disease or cancer in other organs and is not diagnostic for carcinoma of the gall bladder. The following points can be stressed: (1) advanced age, (2) steady dull pain or a change from ordinary biliary symptoms to a more steady pain, (3) weight loss with onset soon after the constant pain, (4) absence of anemia, and later presence of (5) a tumor in the right upper quadrant, and probably jaundice.—J. L. M.

Adenoma solitario del hígado. [Solitary Adenoma of the Liver.] DEL PINO, P., COLILLAS, D., and MASCIOTTRA, R. L. [Buenos Aires, Argentina] *Rev. méd.-quir. de pat. fem.*, **18**:363-379. 1941.

Several cases are reported with a review of the subject. Thirteen photomicrographs are appended.—M. D-R.

Primary Hepatic Carcinoma in Infancy. Report of Two Cases. PLATOU, R. V., and HILL, A. J. [Univ. of Minnesota, Minneapolis, Minn.] *Journal-Lancet*, **62**:191-194. 1942.

Two cases of primary carcinoma (hepatomas, neoplastic hyperplasia of hepatic tissue) occurring in young infants aged 2 and 3½ months.—M. E. H.

Malignant Lesions of the Biliary Tract. ROBERTSON, H. E., SNELL, A. M., and WALTERS, W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:85-91. 1941.

In patients with carcinoma of the biliary passages, the difficulties of management are as much of a problem as those of diagnosis. Four cases of carcinoma in different regions of the extrahepatic biliary tree were presented for discussion. Each illustrated one or more diagnostic and therapeutic problems during the preoperative and postoperative course. Necropsy reports were available.—J. L. M.

Squamous Cell Epithelioma of the Gallbladder: Report of a Case. WIIG, L. M., and CLAGETT, O. T. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:532-534. 1941.

The authors report a case of squamous cell epithelioma of the gall bladder with successful surgical removal in the presence of perforation and peritonitis.—J. L. M.

BONE AND BONE MARROW

Multiple Myeloma. Report of a Case FLAX, J. [Richmond, Va.] *Virginia M. Monthly*, **68**:605-608. 1941.

A case report.—M. E. H.

Neoplastic and Related Conditions in the Bones of Children. GESCHICKTER, C. F. [Baltimore, Md.] *Rhode Island M. J.*, **25**:98-101. 1942.

A short résumé of the benign and malignant neoplastic lesions of the bones of children is presented.—M. E. H.

Orbitoethmoidal Osteoma with Infected Intracranial Mucocele. A Cerebral Abscess of Unusual Origin. HAMBY, W. B. [Univ. of Buffalo Sch. of Med., Buffalo, N. Y.] *Arch. Otolaryng.*, **36**:510-513. 1942.

A case report. It is suggested that correction of the lesion may be easier if diagnostic aspiration of the suspected mucocele is done through a simple trephine before craniotomy is performed.—M. E. H.

Central Chondrosarcoma of the Femur. KENNEDY, R. H. [New York, N. Y.] *Ann. Surg.*, **114**:1106-1110. 1941.

A case report of central chondrosarcoma of the upper femur, nearly 6 years after curettement. Bone Sarcoma Registry #1644.—M. R. D.

Osteogenic Sarcoma Following Open Reduction for Fracture of the Humerus. KIRKMAN, J. H. [Veterans' Administration, Wichita, Kans.] *M. Bull. Vet. Admin.*, **18**:436-437. 1941-42.

A case report that raises the question of the role played by the site of the fracture and the method of treatment in the occurrence of a tumor.—M. E. H.

Plasma-Cell Myeloma of Bone, of Over 12 Years' Duration. KIRSCH, I. E. [Veterans' Administration, Salt Lake City, Utah] *M. Bull. Vet. Admin.*, **18**:96-97. 1941-42.

A case report.—M. E. H.

Malignant Tumors of Bone. MEYERDING, H. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:70-71. 1941.

This is a report on 424 cases of primary malignant tumors of the bone. The tumors fell into the following classes: about 50% were osteogenic sarcoma; about 25%, Ewing's sarcoma; less than 10%, fibrosarcoma; about 10%, multiple myeloma; and less than 2%, giant cell sarcoma (malignant). On less than 2% a clinical diagnosis of sarcoma had been made. The greatest percentage of the tumors (almost a third) occurred in patients between 10 and 19 years of age, and more than a fifth in those between 20 and 29 years; thus the majority of the tumors occurred in patients under 30. Of the 424 patients about a third underwent amputation and about a fifth underwent excision. Following radical surgical treatment, irradiation with or without the administration of Coley's toxin was carried out. In a fourth of the patients biopsy and irradiation were employed; in the remainder treatment was by irradiation alone.

Of the patients who received surgical treatment, 25% were alive after 5 years. Of those who did not receive surgical treatment, slightly less than 10% were alive after 5 years. Although surgical treatment is preferable in the majority of cases, great emphasis is laid on the value of roentgenology in the diagnosis, prognosis, and treatment of malignant tumors of the bone.—J. L. M.

La degeneración sarcomatosa de la osteítis deformante de Paget. [Sarcomatous Degeneration of Paget's Disease]. SCHAJOWICZ, F. [Buenos Aires Sch. of Med., Buenos Aires, Argentina] *Rev. ortop. y traumatol.*, **12**:131-148. 1942.

A review of the 57 cases so far reported in the literature, in which sarcoma developed from bone affected with Paget's disease. The latter can be considered a precancerous condition although malignancy occurs in only a small percentage of cases. Seven illustrations are included.—M. D-R.

Amputation for Osteogenic Sarcoma of Lower End of Femur. Nine-Year Follow-Up of Two Cases. SOLLEY, F. W. [New York, N. Y.] *Ann. Surg.*, **114**:1099-1101. 1941.

A 9 year follow-up of 2 cases of osteogenic sarcoma of the lower end of the femur, treated by amputation. These cases have not been registered with the Bone Sarcoma Registry.—M. R. D.

Neuroepithelioma in the Temporal Bone. Invasion of the Petrous and Squamous Portions, with Extension into the Middle and Posterior Cranial Fossae. TUTA, J. A. [Chicago, Ill.] *Arch. Otolaryng.*, **35**:745-754. 1942.

A case report. A malignant neuroectodermal tumor arising in and diffusely invading the temporal bone, with extension into the middle and posterior cranial fossae, was classified as a neuroepithelioma.—M. E. H.

Sarcoma osteogénico. Osteoblastoma maligno (Brachetto-Brian). [Osteogenic Sarcoma. Malignant Osteoblastoma (Brachetto-Brian).] VALLS, J. E. [Buenos Aires Sch. of Med., Buenos Aires, Argentina] *Rev. ortop. y traumatol.*, **12**:48-107. 1942.

This is the final paper of an extensive review. There are 89 illustrations.—M. D-R.

Osteoid Osteoma. WILE, S. A. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:229. 1942.

A case report.—M. E. H.

Chordoma. WILKERSON, W. W., JR. [Nashville, Tenn.] *Arch. Otolaryng.*, **35**:925-927. 1942.

A case involving an intracranial chordoma. The onset and the rapid development of the symptoms are facts of interest in this case.—M. E. H.

MISCELLANEOUS

Glomus Tumor and Intramuscular Lipoma: Report of Two Cases. HOFFMANN, H. O. E., and GHORMLEY, R. K. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:13-16. 1941.

Two cases are reported. The first concerned a glomus tumor of 24 years' duration, located within the knee joint. The second involved a lipoma that arose within the soleus muscle and was of 9 years' duration. Both tumors were benign and both were, the authors believe, eradicated by surgical intervention.—J. L. M.

Lipomas. HOGUE, P. N. [King County Hosp., Seattle, Wash.] *West. J. Surg.*, **50**:332-338. 1942.

A classification of lipomas is presented with a brief discussion of the various subgroups and their characteristics. While a large majority of lipomas are benign, there are indications that many more are malignant than is suggested by the records.—M. E. H.

Retroperitoneal Sarcoma. KATES, S. R. [Veterans' Administration, Bath, N. Y.] *M. Bull. Vet. Admin.*, **18**:401-405. 1941-42.

A case report.—M. E. H.

How a Tumor Clinic Should Function. KENNEY, J. F. [Memorial Hosp., Pawtucket, R. I.] *Rhode Island M. J.*, **25**:181-182. 1942.

A plan is outlined for a tumor clinic in a small hospital that would serve to educate both the patient and the doctor in cancer work.—M. E. H.

Malignant Tumors in Persons with Cirrhosis of the Liver. PELLER, S. [New York Univ., New York, N. Y.] *Am. J. M. Sc.*, **205**:798-807. 1943.

The postmortem records of 6,596 cases at Bellevue Hospital were analyzed in a study of the relationship between malignant tumors and cirrhosis in man. Cirrhosis was present in 608 cases (9.2%) in the series. The ratio of intrahepatic tumors was several times increased in the cirrhotic patients as compared with noncirrhotic ones. Furthermore, cirrhotic men showed an increased ratio of malignant tumors of the mouth, pharynx, larynx, and esophagus.—J. G. K.

New Phases of Cancer Research. PITTS, H. C. [Providence, R. I.] *Rhode Island M. J.*, **25**:225-229. 1942.

Under the headings of cause, new methods of attack, and treatment, the author discusses in a general way some of the advances that have been made with the study of carcinogenic compounds, radioactive substances, and the search for cytostatic chemicals.—M. E. H.

Cancer in Childhood. RITVO, M., HOUGHTON, J. D., and MCDONALD, E. J. [Boston City Hosp., Boston, Mass.] *Radiology*, **39**:278-282. 1942.

Tables are presented showing death rates from cancer in childhood at various ages up to 15 years, compiled from the United States census figures of 1939, and the relative death rates of children in Massachusetts from cancer and 8 other diseases for various years since 1915. In 1939 childhood cancer deaths in Massachusetts exceeded those attributed to pertussis, pulmonary tuberculosis, measles, diabetes, meningitis, syphilis, scarlet fever, or typhoid.

Seventy-two tumors excluding 11 cases of Hodgkin's disease and 27 of leukemia were observed in children under 15 at the Boston City Hospital in the years 1915 to 1939 inclusive. The most frequent sites were as follows: intracranial, 23; kidney, 13; bone, 11; soft tissues, 6; skin and mucous membranes, 5; eye and orbit, 4. A tabular summary compares the types of tumor found with the series reported by Scotti and by Kellert. In the authors' series the commonest tumors were glioma (medulloblastoma, astrocytoma, and mixed gliomas) Wilms' tumor, osteogenic and Ewing's sarcoma, and miscellaneous soft tissue sarcomas. Malignant tumors in children account for 0.7% of the total cancer death rate and are common enough to warrant careful consideration in differential diagnoses.—C. E. D.

Fifty-Two Proven Cases of Carcinoma of the Pancreas and the Ampulla of Vater: With Special Reference to Fatty Infiltration of the Liver. SCHNEDORF, J. G., and ORR, T. G. [Univ. of Kansas Hosps., Kansas City, Kans.] *Ann. Surg.*, **114**:603-611. 1941.

Thirty-five cases of primary carcinoma of the pancreas and 17 of carcinoma of the ampullary region are reviewed because of similarity of symptoms and amenability to similar surgical treatment in their early stages. Associated fatty infiltration and degeneration of the liver are discussed in relation to their influence on pre- and postoperative management.—M. R. D.

Tumor of Carotid Gland. TWOMBLY, G. H., SIMON, L. G., and STEINBERGER, L. [Memorial Hosp., New York, N. Y., and Norwalk Hosp., South Norwalk, Conn.] *Connecticut M. J.*, **7**:172-175. 1943.

A case successfully treated by surgical removal of the tumor is reported.—M. E. H.

The Rôle of the Pathologist in the Management of Cancer. SIMONDS, J. P. [Northwestern Univ. Med. Sch., Chicago, Ill.] *Illinois M. J.*, **81**:388-392. 1942.

The recognition of the pathologist as a consultant in medicine and surgery can add much of value to the patient and to the cancer clinic team as a whole.—M. E. H.

The Rationale of Radical Surgery for Cancer of the Pancreas and Ampullary Region. WHIPPLE, A. O. [Columbia Univ., New York, N. Y.] *Ann Surg.*, **114**:612-615. 1941.

Personal experience is related concerning the conduct and hazards of pancreaticoduodenectomy in both two and one stage procedures.—M. R. D.